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MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XLIV MARCH–APRIL, 1952 No. 2

HELIXIN, AN ANTIBIOTIC ACTIVE AGAINST CERTAIN FUNGI AND BACTERIA [1]

CURT LEBEN, G. J. STESSEL, AND G. W. KEITT [2]

(WITH 1 FIGURE)

In recent years a number of antibiotics have been studied in relation to the inhibition of plant pathogens and to plant disease control (**5**, **15**). The materials employed in these studies have for the most part been developed with medical rather than phytopathological uses in mind. It seemed desirable, therefore, to seek antibiotics that might be especially suited for plant disease studies, and toward this end a program was initiated for selecting soil microorganisms that produce antibiotics that inhibit plant pathogens and have other desirable characteristics. A result of this work, which has been reported in brief (**12**), has been the selection of several organisms suitable for further study. One of these, an actinomycete from composted soil, designated isolate *A158*, produces the antibiotic that is the subject of this paper. Studies on the identity of this organism and on the production, partial purification, properties, and *in vitro* action of the antibiotic it produces are recorded. Portions of this work have been reported briefly (**7**).

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. Accepted for publication June 6, 1951.

[2] The authors wish to acknowledge the technical assistance of William Whittingham and William Tyler.

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A name, helixin, is tentatively proposed for the antibiotic produced by isolate *A158*, since the antifungal activity and other properties of this material appear to differentiate it from other antibiotics we have noted in the literature. The name was suggested by the helical nature of the spore chains produced by the antibiotic organism.

MATERIALS AND METHODS

The antibiotic organism. The morphology of the antinomycete that produces helixin was studied with slide cultures (**3, 8**), using various media, e.g., Czapek's agar (**14**), nutrient-peptone agar (**13**), glucose-asparagine agar (**14**), and yeast-extract agar (**14**). The mycelium was visible macroscopically after two to three days incubation at 26° C. On most media early growth was first white and later gray; the color change appeared to be associated with the development of dense masses of spores. Spores were formed in four to eight days at 26° by the segmentation of spirals of sporing hyphae (FIG. 1, A) that were usually borne on short lateral branches extending from the aerial mycelium. Spirals were situated typically in dense groups along the main portions of aerial hyphae, especially toward the center of the colony (FIG. 1, C). In the early formation of sporing hyphae spirals tended to be open (FIG. 1, A); they became closed and compact, however, prior to the formation of spores (FIG. 1, B). In late stages these fruiting structures tended to appear ball-like rather than spiral (FIG. 1, B). When water was added to a culture of the organism, spirals and balls broke up into component spores; often several spores were attached end to end in the suspension, and occasionally one loop of a spiral (six to eight spores) was observed. Aerial hyphae were 0.6 to 1.3 μ in diameter. Spores were oval, hyaline, and 0.6 to 1.3 μ wide by 0.7 to 2.0 μ long; the diameter of spore spirals was 4–8 μ. The above description indicates that the organism is a member of the genus *Streptomyces* Waksman and Henrici; it may be placed in Group 4 in Bergey's Manual (**1**)—*spiral formation in aerial mycelium: short, compact spirals.*

To investigate further the identity of the organism it was grown on a number of media that commonly have been used in the study of members of the genus *Streptomyces*. The type of growth, pig-

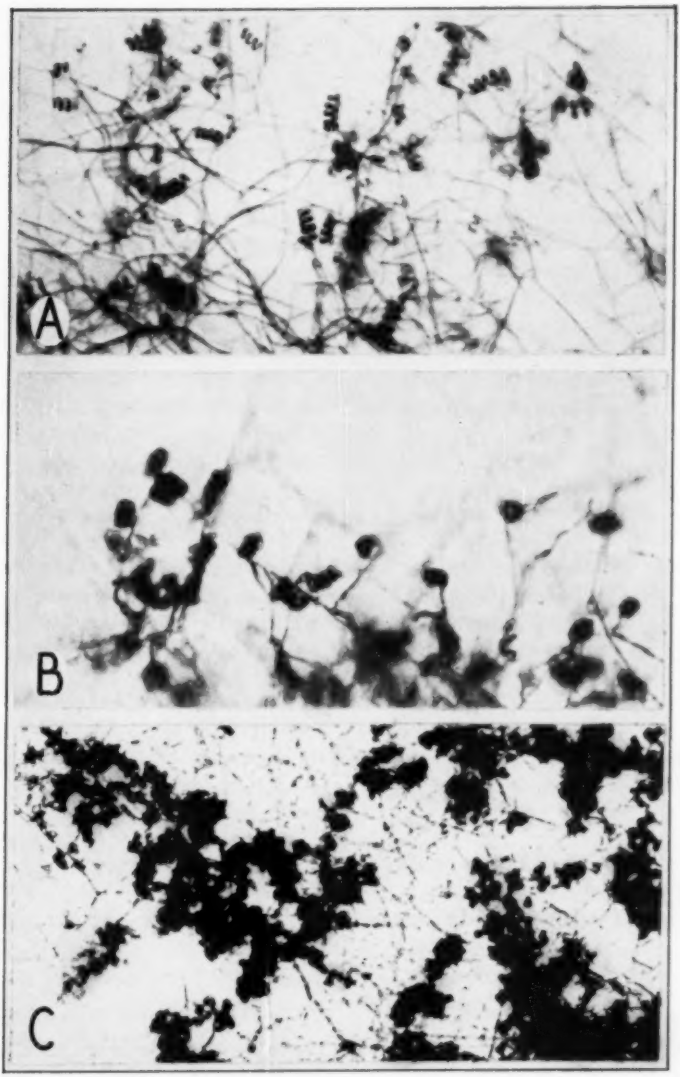

FIG. 1. *The Streptomyces sp. that produces helixin.* A. Loose, spiral, sporing hyphae observed in young cultures. B. Spirals after contraction and segmentation into spores. C. Dense groups of spirals borne on aerial hyphae.

ment production, and other characteristics were noted. Two series of tests were made, each employing duplicate tubes or plates of each medium. Media were seeded by streaking; all cultures were kept at 26° except gelatin cultures, which were kept at 20°. Results are reported for a 15-day incubation period, except as noted. The media used[3] and the results obtained are given below.

1. Czapek's agar (**14**). Abundant, pebbly, light neutral gray, aerial mycelium. No diffusible pigment.
2. Gelatin (**13**). Translucent, cream-colored, vegetative mycelium; sparse, white, aerial mycelium. Slight liquefaction at 15 days, moderate at 30 days. No diffusible pigment.
3. Glucose-asparagine agar (**14**). Results the same as with medium 1.
4. Glucose-peptone agar B (**14**). Results the same as with medium 1.
5. Glucose broth (beef-extract broth (**10**) plus 0.5 per cent glucose). Coalescing, white, powdery colonies on the surface of the medium. Liquid clear, not pigmented. Small amount of flaky sediment present.
6. Malate-glycerin agar (**13**). Abundant, pebbly, light neutral gray, aerial mycelium. Faint green diffusible pigment.
7. Milk, skimmed (**13**). Cream-colored growth ring. Coagulated; peptonized in 15 to 30 days, becoming slightly alkaline. Serum yellowish-orange, slightly turbid.
8. Nutrient-peptone agar (**13**). Cream-colored, shiny, vegetative mycelium; moderate amount of pebbly, white, aerial mycelium. No diffusible pigment.
9. Potato-glucose agar (**14**). Abundant, pebbly, neutral gray, aerial mycelium. Faint, brown, diffusible pigment.
10. Potato plug. Finely wrinkled, cream-colored, vegetative growth with sparse, white, aerial mycelium. Plug darkened slightly.
11. Starch agar (**13**). Abundant, white to neutral gray, pebbly, aerial mycelium. No diffusible pigment.
12. Yeast-extract agar (**14**). Abundant, pebbly, neutral gray, aerial mycelium. No diffusible pigment.

The treatment of starch agar cultures with Lugol's iodine solution (**11**) indicated that the organism possessed weak to moderate

[3] The *p*H of the medium after autoclaving was as follows (two figures given where the *p*H varied in the two series of tests): Medium 1. 7.0, 7.2; 2. 5.4; 3. 7.8; 4. 6.6; 5. 6.7; 6. 6.6; 7. 6.6; 8. 6.0, 6.7; 9. 6.7; 10. 6.3; 11. 6.8; 12. 6.6, 6.9.

diastatic action. Nitrate reduction tests were made with nitrate broth cultures (**10**) (*p*H before seeding, 6.7). The sulphanilic acid-alpha-naphthylamine method (**11**) and a starch-KI-H_2SO_4 method cited by Smith *et al.* (**9**) were used. Tests were made at three and at five days. Traces of nitrite were found by both methods to be produced in five days.

On the basis of the information given in the preceding paragraphs, the organism that produces helixin appears to differ from the spiral-forming *Streptomyces* species described in Bergey's Manual (**1**).

Production of the antibiotic. Screening tests (**12**) showed that isolate *A158* was antagonistic to each of eight test fungi and to one of two bacteria when grown in the same agar culture plate. It was also shown that isolate *A158* produced an antibiotic that inhibited one of the test fungi, *Glomerella cingulata* (Stoneman) Spauld. & v. Schrenk, when grown in shaken liquid media containing various natural products. The greatest production of the antibiotic was found to be in a soybean meal-glucose medium,[4] and this was used in all experiments reported in this paper. A maximum antibiotic production of 2.5 to 5.0 units (u. defined below) per ml. was achieved in six to eight days; during this time there was a change of *p*H from 7.0 to 7.9–8.3. Preliminary tests with small tank fermentations (**2**)[5] indicated that this method of culture had the advantage of yielding more of the antibiotic in a shorter time than when shaken flasks were used. Five to 10 u. per ml. were obtained in four days using lard oil as the antifoam agent.

The assay. A microbiological assay employing the fungus *Glomerella cingulata* was used to determine the antibiotic potency of test preparations. Blotting paper disks containing the preparation were placed on the surface of seeded agar in Petri plates, and the size of the resulting inhibition zones around the disks was noted after an incubation period. The zone size produced by a given preparation being known, the antibiotic concentration of this prep-

[4] Gms. per liter: soybean extractor meal, 40; glucose, 20; $CaCO_3$, 1.5. Antibiotic production varied with different meals; best results were obtained with a 76 per cent water-soluble protein meal obtained from Archer-Daniels-Midland Co., Minneapolis, Minnesota.

[5] We are indebted to W. H. Peterson and M. J. Johnson for their counsel and for the use of fermentation facilities.

aration was determined by reference to a dosage-response curve drawn on the basis of the size of inhibition zones produced by known concentrations of a standard preparation.

The method of seeding and the medium were the same as for antimycin A (**4**). Antibiotic preparations were dissolved in ethanol for assay. Details concerned with the dilution of samples, the placement and size of disks, and the calculation of potency were the same as for antimycin A (**4**); instead of an incubation period of 40–48 hours, however, an incubation period of 24–30 hours was used. For the standard, a "crude ethanol helixin" solution (see "Fractionation procedures") was arbitrarily assigned a potency of eight u. per ml., and each time an assay was set up the standard was tested at one, two, four, and eight u. per ml.[6]

The zone diameter was found to be a straight line function of the log of the antibiotic concentration, and dosage-response curves produced by test preparations were usually parallel to that of the standard. Occasionally activity of a sample was determined without using dilutions by interpolation of the standard curve. Culture filtrates were assayed after dilution with four volumes of ethanol.

Fractionation procedures. With the aid of the assay, various methods were tried for purifying the antibiotic and ultimately a fractionation procedure was devised. A sample fractionation follows: 1) Seven cultures (two-liter Erlenmeyer flasks each containing 400 ml. of medium; each flask seeded with 10 ml. of a culture shaken eight days at 26°) shaken eight days at 26° in a reciprocating shaker (66, 15 cm. strokes per min.). 2) Cultures centrifuged, sediment discarded. 3) Centrifugate (7700 u. in 1900 ml.; 0.125 u. per mg. dry weight) *p*H changed from 7.9 to 3.0 (HCl), centrifuged, and inactive centrifugate discarded. 4) Sediment extracted twice with ethanol and extracts pooled (1100 ml., 0.85 u. per mg.); ethanol insoluble material discarded. 5) Extract, which contained some water from the sediment and which hereinafter is called "crude ethanol helixin," taken to 120 ml. (reduced pressure) and the resulting aqueous ethanol mixture extracted three times with equal volumes of chloroform; active dark red gum

[6] One u. per ml. usually produced inhibition zones 16–22 mm. in diameter. There appeared to have been no decomposition of the standard in nine months at 8°.

on extraction flask and at the solvent interface collected; inactive aqueous ethanol and slightly active chloroform phases discarded. 6) Red gum taken up in 250 ml. of 50 per cent ethanol; inactive precipitate discarded. 7) Fifty per cent ethanol solution taken to 110 ml. (reduced pressure) and then discarded; red gum on flask taken up in 200 ml. of absolute ethanol and inactive precipitate discarded. 8) Active deep red ethanol solution made to 250 ml. (3500 u., 2.75 u. per mg.). This procedure resulted in a 22-fold purification, and approximately one-half of the original activity was accounted for in the final product. Preparations of similar purity have also been obtained by the following modified procedure: 1) "Crude ethanol helixin" diluted with an equal volume of water. 2) Resulting suspension filtered, precipitate discarded. 3) Filtrate concentrated (reduced pressure) to one-half original volume of "crude ethanol helixin" and discarded. 4) Red gum that separated out during concentration collected in absolute ethanol. 5) Inactive precipitate discarded; active ethanol solution a deep red.

Solubility, stability, and other tests were made with ethanol solutions containing 2.5–2.7 u. per mg. To test solubility, one volume of the solution was taken to dryness (reduced pressure) and from five to ten volumes of the test solvent added. As determined by observation and by assay of the dissolved and undissolved material, the antibiotic was indicated to be soluble in ethanol, methanol, methyl cellosolve, pyridine, 0.01 M phosphate buffer at *p*H 8.5, and glacial acetic acid; it was slowly soluble in *n*-butanol, partly soluble in acetone, slightly soluble in chloroform, and insoluble in ether, petroleum ether, benzene, ethyl acetate, and carbon tetrachloride. When alkaline aqueous solutions were made more acid than *p*H 4–5, an active amorphous precipitate was formed; this could be redissolved by making the solution alkaline. Aqueous solutions at *p*H 7.0 gave negative Molisch, ninhydrin, Hopkins-Cole, xanthoproteic, Millon, and $FeCl_3$ tests. Stability at 100° was tested at *p*H 3.0, 7.3, and 9.0; after 16 minutes the approximate activity remaining at the three *p*H levels was found to be, respectively, 0, 45, and 65 per cent. Screening tests with the culture filtrate adjusted to *p*H 6.0 had indicated prior to these tests that approximately 50 per cent of the activity remained after the filtrate had been autoclaved 15 min. at 15 p.s.i.

Agar-streak tests. The effect of partly purified helixin on a number of microorganisms was determined with the aid of agar-streak tests. In these tests varying amounts of the antibiotic were incorporated into an agar medium, which was then streaked with suspensions of test organisms. After a suitable incubation period the inhibition point for each organism was taken as the smallest amount of the antibiotic that prevented macroscopic growth.

The methods and materials used in the tests have been described previously (**6**). Suspensions for seeding all phytopathogenic bacteria, the yeast, and all fungi except *Venturia inaequalis* (Cke.) Wint. were prepared from potato-glucose agar [7] cultures that had grown four to seven days at 22–24°. That for *V. inaequalis* was made from five-week-old cultures kept at 16° on potato agar.[8] Suspensions of the other bacteria were prepared from nutrient agar [9] cultures grown 24 hours at 37°. The antibiotic was incorporated in potato agar [8] for tests with fungi, phytopathogenic bacteria, and the yeast, and in nutrient agar [9] for tests with the bacteria grown at 37°. The antibiotic preparation (2.7 u. per mg.) was carried in ethanol; suitable controls showed that at the concentrations used ethanol had no inhibitory effects. The *p*H of the potato agar was 5.9, and the *p*H of the nutrient agar was 6.2. The addition of the antibiotic did not change the reaction of either medium.

Data obtained in the agar-streak experiments are given in TABLE I. It will be noted that all fungi were completely inhibited by the antibiotic at 15 μg. per ml. or less, whereas some bacteria were not inhibited by 60 μg. per ml. The table is based on the amount of antibiotic necessary to produce complete inhibition; inhibitory effects, as indicated by sparse or atypical growth of the test organisms, were noted at smaller concentrations. For example, *V. inaequalis* with an inhibition point of 0.47 μg. per ml. was observed to be partly inhibited at 0.11 μg. per ml.

[7] Per liter: extract from 200 gms. of sliced, peeled potatoes steamed one-half hour in 500 ml. of water; glucose, 25 gms.; agar, 17 gms.

[8] Per liter: extract from 40 gms. of sliced, peeled potatoes steamed one-half hour in 500 ml. of water; glucose, 5 gms.; agar, 17 gms.

[9] Gms. per liter: beef extract, 3; peptone, 10; agar, 17.

TABLE I

INHIBITION OF VARIOUS MICROORGANISMS BY PARTLY PURIFIED HELIXIN IN AGAR-STREAK TESTS

Organism	Incubation		Inhibition point[a]
	Time	Temperature	
	Hrs.	°C.	μg. per ml.
Agrobacterium tumefaciens (E. F. Sm. and Towns.) Conn	60	24	>60.00
Alternaria solani (E. and M.) Jones and Grout	60	24	1.88
Bacillus subtilis Cohn *emend.* Prazmowski	24	37	30.00
Botrytis allii Munn	60	24	1.88
Candida albicans (Robin) Berkhout	60	24	7.50
Colletotrichum circinans (Berk.) Vogl.	60	24	3.75
C. phomoides (Sacc.) Chester	60	24	3.75
Fusarium oxysporum f. *lycopersici* (Sacc.) S. and H.	60	24	7.50
F. oxysporum f. *pisi* (Linford) S. and H.	60	24	15.00
Glomerella cingulata (Stoneman) Spauld. and v. Schrenk	60	24	3.75
Helminthosporium sativum Pam., King, and Bakke	60	24	0.93
H. victoriae M. and M.	60	24	3.75
Micrococcus pyogenes var. *aureus* (Rosenbach) Zopf	24	37	15.00
Nigrospora sphaerica (Sacc.) Mason	60	24	0.93
Penicillium notatum Westling	60	24	7.50
Proteus vulgaris Hauser	24	37	>60.00
Pseudomonas aeruginosa (Schroeter) Migula	24	37	>60.00
Saccharomyces cerevisiae Meyen	60	24	7.50
Sclerotinia fructicola (Wint.) Rehm	60	24	1.88
Stemphylium sarcinaeforme (Cav.) Wilts.	60	24	3.75
Trichophyton mentagrophytes (Robin) Blanchard	96	24	3.75
Venturia inaequalis (Cke.) Wint.	240	16	0.47
Verticillium albo-atrum Reinke and Berth.	60	24	0.93
Xanthomonas campestris (Pammel) Dowson	60	24	15.00
X. phaseoli (E. F. Sm.) Dowson	60	24	>60.00

[a] The smallest amount (dry weight) that completely inhibited macroscopic growth. Each entry is based on two series of tests made at different times except that for *V. inaequalis*, which was tested once.

DISCUSSION

Like actidione, antimycin, glutinosin, and others, helixin appears to be an antibiotic potent primarily against fungi. Most of the fungi tested are plant pathogens, but it is noteworthy that two organisms pathogenic to man, the yeast-like fungus, *Candida albicans*, and *Trichophyton mentagrophytes*, which is associated with the athlete's foot disease, were also inhibited by small amounts of the partly purified material.

In the present paper as a matter of convenience the active material has been implied to be a single substance. The possibility is

recognized that in the crude material there may be more than one antibiotic, and work is at present under way to investigate this possibility and to isolate the active material or materials. These efforts, as well as further investigation of the partly purified material in various other aspects, including plant disease control, appear to be warranted in view of the wide range of activity of the antibiotic to important plant and animal pathogens.

SUMMARY

The production, partial purification, properties, and *in vitro* activity of an antibiotic, produced by a *Streptomyces* sp. and tentatively designated helixin, are described. In agar-streak tests the partly purified antibiotic was found to inhibit completely all fungi studied, including pathogens of plants and man, in the range of 0.47 to 15 μg. per ml. It also inhibited a yeast and some of the bacteria tested.

NOTE

After the manuscript for this paper was completed for publication, the article by David Gottlieb, P. K. Bhattacharyya, H. E. Carter, and H. W. Anderson entitled "Endomycin, a new antibiotic" appeared in Phytopathology, volume 41, pages 393–400, 1951. Endomycin and the *Streptomyces* sp. that produces this antibiotic have similarities to helixin and to the organism that produces it. Further studies are desirable to ascertain the degree of similarity between the two antibiotics and the two organisms.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

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EFFECT OF ACTIDIONE ON MOLD CONTAMINANTS AND ON HUMAN PATHOGENS [1]

CÉSAR A. FUENTES,[2] FERNANDO TRESPALACIOS,[3] GUILLERMO F. BAQUERO,[3] AND RAQUEL ABOULAFIA [2]

The isolation of pathogenic fungi from clinical materials is often prevented by an overgrowth of bacteria and saprophytic molds. Media which have been developed to inhibit the growth of bacteria are of aid in the isolation of fungi, but the selecting isolation of pathogens from among rapidly growing saprophytes has presented a more difficult problem.

Many antibiotics have been tested against fungi, but very few have shown any activity against them. One such agent, fradicin (**4**), has been shown to be inhibitory to the following species: *Penicillium notatum, Aspergillus niger, Ceratostomella ulmi, Trichophyton mentagrophytes* and *Candida albicans.*

Recently the discovery of the antibiotic actidione, isolated from cultures of *Streptomyces griseus* by Leach, Ford and Whiffen (**2**), has brought forth a promising new tool for the isolation of pathogenic fungi from materials contaminated with saprophytic fungi. Whiffen (**5**) demonstrated that while actidione has no antibacterial activity, it is active against a large number of yeasts and a few species of the pathogenic fungi. Phillips and Hanel (**3**) described its use for the isolation of bacteria artificially contaminated with saprophytic fungi, and Georg, Ajello and Gordon (**1**) have suggested its employment for the isolation of *Coccidioides immitis* from clinical specimens, or from the physical environment.

[1] We are indebted to Dr. V. Pardo Castello, Chief of the Department of Dermatology and Syphilology, Calixto Garcia Hospital, for the facilities given in the accomplishment of this work.

[2] Department of Experimental Pathology, University of Havana, School of Medicine, Havana, Cuba.

[3] Department of Dermatology and Syphilology, University of Havana, School of Medicine, Havana, Cuba.

The purpose of this study was to observe the action of actidione on saprophytic and pathogenic fungi obtained from human sources, as well as on various strains of fungi maintained in our culture collection.

MATERIALS AND METHODS

Actidione was furnished through the courtesy of the Upjohn Co., Kalamazoo, Michigan. Clinical specimens for culture were obtained from patients of the Department of Dermatology and Syphilology, Calixto Garcia Hospital, University of Havana, School of Medicine. Stock cultures were derived originally from the following sources: Communicable Disease Center, Atlanta; Oswaldo Cruz Institute, Rio de Janeiro; and the mycology laboratory of The Jewish Hospital, Cincinnati. All cultures have been maintained in this laboratory under mineral oil.

Material from lesions suspected of being mycotic in origin was planted, usually in duplicate or triplicate, on tubes of Sabouraud dextrose agar (Bacto-peptone 10 g.; dextrose, Merck C. P. 40 g.; Difco agar 20 g.; and distilled water up to one liter), and on the same medium to which 0.1 mg. actidione per ml. had been added. The actidione was prepared in distilled water solution, filtered through ST pads [4] and added to the autoclaved medium, which had been cooled to 45° C. No antibiotics were added to inhibit bacterial growth. All media were freshly made, and clinical materials were not treated with alcohol previous to planting. Culture readings were made at the end of ten days, and microscopic examinations were made in all except typical *T. rubrum* colonies.

RESULTS

A comparison of the growth on the Sabouraud-actidione and plain Sabouraud media of the organisms, both bacterial and fungal, obtained from the lesions cultured is presented in TABLE I. An analysis of TABLE I shows that in this series of 20 patients the isolation of pathogenic fungi was about equally efficient on both media, 11 and 10 strains respectively being recovered. However, fewer saprophytic fungi developed on the actidione medium. Only 3 tubes of the latter were contaminated with saprophytes, while

[4] Hercules Filter Corp., Paterson, N. J.

8 tubes of the Sabouraud agar were covered with their growth. The two media supported growth of bacteria equally well.

TABLE II shows that of 42 pathogenic stock strains, all except 6 grew well on the actidione medium. Of these, one was *Cryptococcus neoformans,* which had been shown by Whiffen (**5**) to be

TABLE I

EFFECT* OF ACTIDIONE IN THE ISOLATION OF PATHOGENIC FUNGI FROM CLINICAL MATERIALS

Clinical diagnosis	Sabouraud-actidione medium	Sabouraud agar
Eczema marginatum	*Trichophyton rubrum* +, Bacteria	*T. rubrum* +, Bacteria
Eczema marginatum	*T. rubrum* +	*T. rubrum* +
Eczema marginatum	Bacteria	*T. rubrum* +, Bacteria
Eczema marginatum	*T. rubrum*	Bacteria
Tinea pedis	Bacteria	*Epidermophyton floccosum*
Tinea pedis	*T. mentagrophytes* ++	*T. mentagrophytes* ++, *Aspergillus niger*, *A.* sp. and *Mucor* sp.
Tinea pedis	*E. floccosum*	Bacteria
Tinea capitis	*T. tonsurans* +, Bacteria	*T. tonsurans* +, Bacteria
Tinea capitis	*Microsporum canis* ++	*M. canis* ++
Tinea capitis	*M. canis* ++	*M. canis* ++, *Helminthosporium* sp.
Tinea corporis	*M. canis* ++	*M. canis* ++
Chromoblastomycosis	*Hormodendrum Pedrosoi* ++, Bacteria	*H. Pedrosoi* ++
T. corporis	*M. canis* ++	No growth
Onychomycosis (Hand)	Bacteria	*Aspergillus glaucus*, *A.* sp.
Squamous epidermophytosis	Bacteria	*Helminthosporium* sp.
Allergic eczema	Bacteria	*Fusarium* sp.+, Bacteria
Lepromatous leprosy	*Penicillium* sp. +, Bacteria	*Penicillium* sp. +, Bacteria
Erythematous vesicular eczema	Bacteria	*A. niger*
Vesicular epidermophytosis	*Penicillium* sp. +, Bacteria	*P.* sp. +, Bacteria
Onychomycosis and paronychia	*Penicillium* sp. ++	*P.* sp. ++, *A.* sp. ++

* + = poor growth, ++ = fair growth, +++ = good growth, ++++ = excellent growth.

sensitive to this antibiotic. *Paracoccidioides tenuis* did not grow on the actidione medium and its development on Sabouraud agar was poor.

In general, the actidione medium did not exert any appreciable inhibitory action on the growth of most of the pathogenic fungi

isolated from the patients and did hold down saprophytic contamination to some extent. Slow growing organisms such as *Trichophyton faviforme, Nocardia madurae* and *Histoplasma capsulatum* behaved as usual. *H. capsulatum* was isolated on both media from material taken from an inguinal gland of a patient with histoplasmosis, but in 8 days, growth on the actidione medium was not as profuse as on the Sabouraud medium. However, after 10 days no difference in amount of growth could be detected and microscopic examination showed no morphologic alteration of the tuberculate chlamydospore. Yet plantings on Sabouraud Actidione

TABLE II

COMPARATIVE GROWTH* ON SABOURAUD-ACTIDIONE MEDIUM AND SABOURAUD AGAR OF PATHOGENIC AND SAPROPHYTIC FUNGI WHICH HAD BEEN MAINTAINED UNDER MINERAL OIL

Organism	Sabouraud-actidione medium	Sabouraud agar
Blastomyces dermatitidis	++	++
Candida Guilliermondi 2694	++	++++
C. stellatoidea 2567	++	++++
C. tropicalis 2689	++	++++
Cryptococcus neoformans	No growth	+
Microsporum Audouini	+++	++++
M. ferrugineum 569	+	+, *Helminthosporium* sp.
M. gypseum 207	++	+++
Histoplasma capsulatum 2752	+	++
Hormodendrum Pedrosoi 1885	++	++, *Penicillium* sp.
Monosporium apiospermum 2744	++	++++
Nocardia madurae 2593	+	++
Paracoccidioides cerebriformis 2649	+, *Penicillium* sp. ++	No growth, *P.* sp. ++++
P. tenuis 2648	No growth	+
Phialophora verrucosa 867	++, *Aspergillus* sp. +	No growth, *A.* sp. ++++
P. verrucosa	++	++
Piedraia Hortai	+	+
Trichophyton concentricum 2741	+	++
T. Megnini 2684	++	++
T. mentagrophytes A 311	+++	+++
T. mentagrophytes 414	+++	No growth, *Cephalosporium* sp. +++
T. mentagrophytes A 314	+++	+++
T. mentagrophytes 418	+++	+++, *A.* sp. +++
T. mentagrophytes A 213	+++	+++
T. mentagrophytes A 219	+++	+++
T. mentagrophytes B 44	+++	+++
T. mentagrophytes A 310	+++	+++

* + = poor growth, ++ = fair growth, +++ = good growth, ++++ = excellent growth.

TABLE II—*Continued*

Organism	Sabouraud-actidione medium	Sabouraud agar
T. mentagrophytes A 143	+++	+, *A.* sp. ++
T. mentagrophytes 2722	++	++
T. rubrum A 206	++++	+++, *A.* sp. +++
T. rubrum B 11	+++, *A.* sp.	No growth, *P.* sp. ++++
T. rubrum B 83	+++	+, *A.* sp. +++
T. rubrum 1823	+++	+++
T. rubrum B 4	++, *A.* sp.	No growth, *A.* sp. ++++
T. rubrum	+++	+++
T. Sabouraudi 2668	++	++
T. tonsurans A 211	+++	+++
T. tonsurans 2739	++	++
T. violaceum 496	++	++
T. violaceum 2661	+	+
T. epilans (cerebriformis) 1470	++	++
Achorion Schoenleini 1763	+	No growth, *A.* sp. ++
Trichosporon Beigelii 272	+	+
Trichosporon Beigelii 2633	++	+++
Saprophytes		
Aspergillus fumigatus A 258	+++	++++
A. glaucus 235	++	++++
Alternaria sp. A 256	+++	++++
Cryptococcus sp. 144	No growth	+
Fusarium sp. A 93	++	++++
Helminthosporium 221	No growth	++++, *A.* sp. +
Mucor sp. A 281	+++	+++
Nigrospora sp. 67	No growth	++++
Paecilomyces sp. 383	No growth	++++
Penicillium sp. 225	++	++++
Penicillium sp. 243	++	++++
Rhizopus sp. 259	++	++
Rhodotorula sp. 296	No growth	+++
Syncephalastrum sp.	+++	++++
Trichoderma sp. 236	No growth	++++

400 micrograms of squeezed organs of mice inoculated with the same strain of *H. capsulatum* did not grow at all, while those plantings on Sabouraud dextrose did grow. It is interesting to note that *Hormodendrum Pedrosoi,* the etiologic agent in a case of chromoblastomycosis, was isolated on both media.

Of the 15 stock saprophytic fungi transferred to these two media, 8 showed slower and more restricted growth on the actidione medium and 6 strains: *Cryptococcus* sp., *Helminthosporium* sp., *Paecilomyces* sp., *Rhodotorula* sp., and *Trichoderma* sp., did not grow at all.

These results were to be expected since many saprophytic fungi are inhibited by actidione.

SUMMARY

1. Pathogenic fungi were isolated from clinical lesions as readily on actidione medium as on Sabouraud's agar.

2. Cultures made on actidione medium were less frequently contaminated with saprophytic fungi than were cultures on Sabouraud's agar.

3. Of 42 stock cultures of pathogenic fungi, all except 6 were shown to grow well on the actidione medium.

4. Of 15 stock cultures of saprophytic fungi, the growth of 8 strains was considerably less on the actidione medium than on the Sabouraud medium, while six strains did not grow at all on the former.

5. The actidione medium caused no change in the gross or microscopic characteristics of the cultures studied.

6. The actidione medium may be useful in the isolation of pathogenic fungi from clinical lesions, and may aid in keeping stock cultures free from contamination with saprophytic fungi.

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KERATIN DIGESTION BY DERMATOPHYTES: A SPECIFIC DIAGNOSTIC METHOD[1]

R. VANBREUSEGHEM

(WITH 1 FIGURE)

A recent work published by R. M. Page (**1**) in MYCOLOGIA confirmed some observations of mine on keratin digestion by dermatophytes (**2, 3, 4, 5**). Strains of *Microsporum gypseum* and some other dermatophytes were grown on agar enriched with horn, nails, wool or human hair. Page observed that dermatophytes are able to digest those products of the skin and he tried to explain how they do it. But we feel from our own observations that he missed the point owing to his technic.

Two years ago we introduced a new technic based on the culture of the dermatophytes on a single hair—human or animal. The method is very simple and it is worthwhile to describe it in detail because it can be used very satisfactorily in other fields of mycology. A hair is stretched from the tip of an S-shaped glass rod to its base and is fixed in this position by collodion. The base of the S-shaped rod forms a triangle on which the rod is supported. On the medium part of the hair a very small piece of a young colony of dermatophyte, grown on Sabouraud's medium, is fixed—by simple contact—and the apparatus is carefully put in the bottom of a jar containing some water. The hair does not come in contact with the water. The jar is closed by a lid and put in the incubator at 25° C. or maintained at room temperature. Usually we use a single jar (15 cm. × 7.5 cm.) for three S-shaped rods.

After some days—from five to ten—the hair is cut with scissors above and under the small colony, put on a slide and examined in chloral-lactophenol under a cover slip. It appears clearly from this

[1] This investigation was made possible by financial assistance from the I.R.S.A.C. (Institut pour la Recherche Scientifique en Afrique Centrale), Director: P. A. Dubois.

examination that the dermatophyte has been growing from its original support along the hair, up and down. If the dematophyte produces normally microconidia and macroconidia, those are beautifully developed and the preparation is one of the best to give a very clear idea of the morphology of the fungus.

But, moreover, most dermatophytes attack the hair and, so doing, they may go so far as to break it completely. The rupture of the hair is the result of two different processes which work separately or in association.

1) One of the processes is generally encountered with *Trichophyton rubrum:* the cuticle of the hair disappears, the cortex is progressively attacked and the thickness of the hair is so much reduced that just before the break the figure is that of two pencils opposed by their tapering ends. It happens too that the dissolving action of the fungus does not go so far as to break the hair; all that one can see is a light erosion of the hair. Those facts prove, in contradiction to the opinion expressed by Page, that the action of the dermatophytes on the hair is not the obligate consequence of the penetration of a hypha of the fungus into the hair; it will be clear enough to anyone examining such a preparation that the cuticle of the hair is attacked by simple contact with the hyphae.

2) The second process is quite different; some of the hyphae after they have progressed for some length along the hair change abruptly their direction and penetrate into the hair forming nearly a right angle. This penetration is perpendicular to the length of the hair. The hypha continues to progress inside of the hair and in so doing perforates it. To those highly specialized hyphae we gave the name of "organes perforateurs" (perforating organs) while Page, later, named them "intrusions." At the starting point, the perforating organ is just like a cone whose base is turned towards the cuticle while the point, or growing tip, progresses in the direction of the centrum of the hair or, more precisely, in the direction of a point situated at the opposite of its entrance into the hair.

Sometimes, for some unknown cause, the perforating organ grows faster than it progresses. Perhaps the hair opposes a greater resistance than usual to its progression or the keratinolytic power of the fungus is reduced. Whatever the cause, the perforating

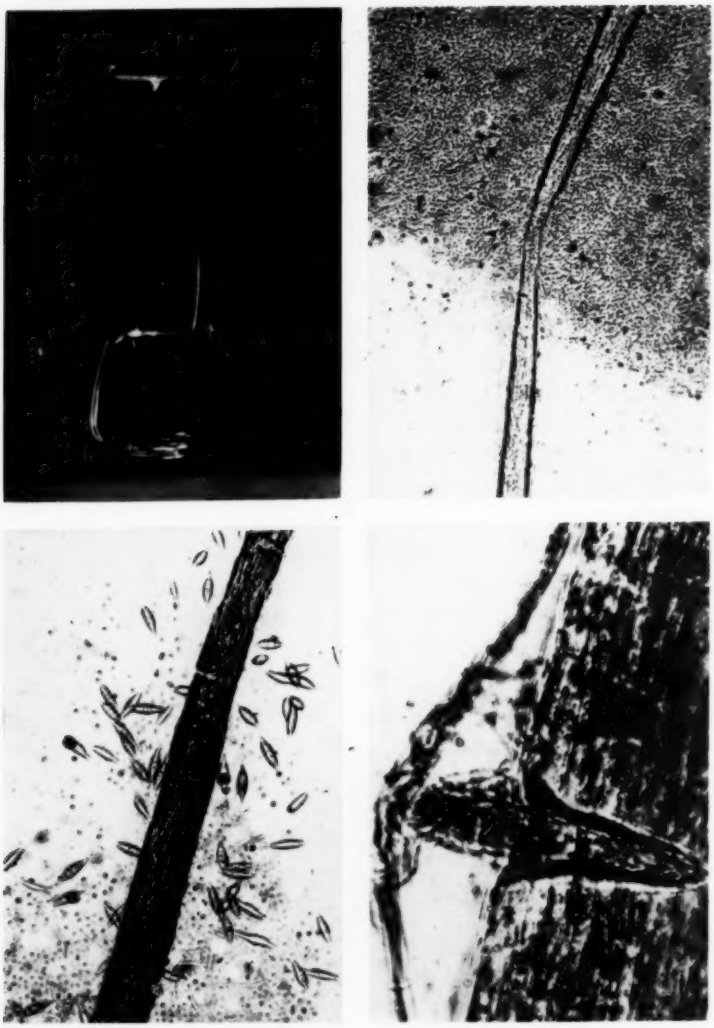

FIG. 1.

Upper left. S-shaped glass rod with human hair. ½ size.

Upper right. *Trichophyton rubrum*. Lesions of the human hair after 17 days. ×70.

Lower left. *Sabouraudites* (*Microsporum*) *canis* on human hair after 16 days.

Lower right. Perforating organ produced on human hair by *Ctenomyces persicolor* after 16 days. ×725.

organ being stopped in its progression must find some place to develop. The result of this is a backward movement at the point of entrance and the formation of a handle-like figure ("anse") over this very point. To this figure which he does not explain, Page gave the name of "eruption." This handle-like figure is really produced by the growth of the perforating organ inside of the hair and not by the hypha from which the perforating organ takes its origin. The proof of this is to be found in the presence on the "anse" of melanic pigments. Those pigments originate—and they cannot have another origin—from the medulla and they are carried out of the hair by the perforating organ in its backward movement.

The perforating organs and the handle-like figure they produce create different figures according as they are viewed from the side or the front. The side view is the one we have just described. Seen from the base or from the tip, they make a round or oval figure on the surface of the hair.

It is evident from the observations that the dermatophyte grows on the hair in vitro very differently from in vivo. In the first case it has all the characters of a saprophyte; in the second case it shows no more than the monotonous morphology induced by the parasitism.

Page says that as soon as the dermatophyte is inside of the hair it grows hyphae parallel to the long axis of the hair as it does *in vivo*. As a matter of fact, it is only when the hair has been disintegrated and lacerated by the enzyme produced by the dermatophyte that the hyphae grow between the fibrils of the hair.

We repeated these experiences with a large variety of different strains. In the genus *Ctenomyces* (Sabouraud's Trichophytons Microïdes or Emmons' *T. mentagrophytes*) we could observe a good keratolytic activity for the species *Ct. asteroïdes, Ct. interdigitalis, Ct. persicolor*. Among the *Sabouraudites* (*Microsporum*), *S. audouini, S. canis, S. gypseus, S. langeroni;* and in *Trichophyton, T. violaceum, T. rubrum, T. schoenleini, T. quinckeanum*, and many others are very active. The only species in the genus *Epidermophyton, E. floccosum* and the only one in the genus *Langeronia, L. soudanensis* (3) are keratolytic. Actually, we observed the action of more than a hundred different strains of dermatophytes and we conclude that every dermatophyte may attack

the hair. Some, nevertheless, are very active and others less so. We can now try to locate the causes of this keratolytic power.

Age of the strain is indifferent; some isolates tested on the hair at their first passage from true parasitic to saprophytic life are very poor keratin destroyers while strains kept in laboratory for forty or fifty years and having lost most of the freshness of their prime, attack the hair vigorously.

What seems to us to play the most important role is the richness in spores, microconidia or macroconidia. The *Ctenomyces*, for instance, and *Epidermophyton floccosum* are always very active. In *Microsporum* the distinction is clear between the so-called animal strains, e.g. *S. canis*, and the human ones, e.g. *S. audouini*, the first one being extremely active while the second is a poor hair destroyer. In *Trichophyton*, *T. quinckeanum* and *T. rubrum*, whose mycelium abounds in microconidia, attack the hair with the utmost vigour, while *T. violaceum*, *T. ferrugineum*, *T. schoenleini*, i.e. the strains with glabrous colonies practically devoided of any spores, have a very poor keratolytic activity.

Thus the keratolytic power seem to run with the richness of each species in spores.

Nevertheless, it sometimes happens that a dermatophyte cultivated on hair in vitro does not attack the support, but this is an exception and if one repeats patiently the test one will generally be able to observe the typical lesions we have described.

It is easy to prove by experimentation that the origin of the hair is indifferent and the same results are produced with hairs from males or females, from adults or from children. Bleached or dyed hairs do not oppose the action of the dermatophytes.

If from the human we pass to other species of animals, we can see that the dermatophytes act on animal hairs in the very same manner as they do on human hair. We used, to prove this, hairs taken from the following animals: *Anoa depressicornis* Smith, *Bos indicus* L., *Bos taurus* L., *Bubalus bubalis* L., *Camelus bactrianus* L., *Camelus dromedarius* L., *Canis familiaris* L., *Capra hircus* L., *Equus asinus* L., *Felis leo* L., *Hyaena hyaena* L., *Lama glama* L., *Ovis aries* L., *Pan Schweinfurthii* Giglioli, *Phacochoerus aethiopicus* Pallas, *Potphagus grunniens* L., *Sus scrofa* L., *Syncerus nanus* Boddaert, *Tragelaphus gratus* Sclater, *Tragelaphus spekii* Sclater,

Ursus arctos L. Inoculated on the hairs of these animals the dermatophytes develop beautifully and produce perfect perforating organs. It is amazing to see that such a common dermatophyte as *Sabouraudites* (*Microsporum*) *canis*, whose keratolytic action on human hair is one of the most powerful, is also able to cut in a few days a wild boar's hair whose diameter is 1 mm. or more. When a perforating organ, after having grown its way through the cortex, falls into the large medulla, melanin laden, which is rather common in the hair of some animals (e.g. lion's hair), the perforating organ, instead of going through the hair perpendicular to the length of the hair, grows in the medulla and forms hyphae; during the development of these hyphae in the medulla the melanin disappears.

We did not experiment on horn or nails but the observations made by Page prove that these substances are, as well as the hair, attacked by the dermatophytes. But we repeated our observations on feathers from some birds: *Branta ruficollis* Pallas, *Coscoroba coscoroba* Molina, *Coturnix coturnix* L., *Dichoceros bicornis* L., *Euodice cantans* Gmelin, *Larus canus* L., *Lophortyx californica* Shaw, *Megalocornis antigone* L., *Pavo cristatus* L., *Tragopan satyrus* L., *Tyto alba* Scopoli. The technic was different but based on the same principle—inoculation of a small piece of a dermatophyte's colony directly on feather. Some barbs of the feather are fixed by collodion on a slide. This is then put in a Petri dish upon a U-shaped rod and some water is placed in the bottom. After some days in the incubator (25° C.) the feather is found to be broken at the very place where the dermatophyte has been inoculated. So the action of dermatophytes on feathers is similar to their action on hairs but we have not been able to see how the section of the feathers is made. The keratinolytic action of the dermatophyte and the invasion of the feather by numerous hyphae were evident: but how this process begins is not clear and we were unable to see any perforating organs.

The last point is important. Are the non-dermatophyte fungi able to attack the hair? We think from the many observations we made that the dermatophytes are the only ones able to attack the hair in the manner we described. About thirty different fungi, most of them from the Centraal Bureau voor Schimmelcultures, in

Baarn, and some of our own collection, were given a trial. None of them attacked the hair although their morphology was perfectly developed on the hairs after a two months' observation. The names of these fungi follow: *Acremonium potroni, Allescherya boydii, Alternaria tenuis, Aspergillus niger, Aspergillus quadrilineatus, Bauveria globulifera, Candida albicans, Cephalosporium acremonium, Chaetomium globosum, Fusarium solani, Glenospora clapieri, Mucor mucedo, Myrothecium verrucaria, Penicillium notatum, Penicillium solitum, Phoma mali, Piedraia hortai, Polyporus vaporarius, Pullularia pullulans, Pythium de Baryanum, Rhizoctonia solani, Sclerotinia fructigena, Scopulariopsis brevicaulis, Sordaria fimicola, Sporotrichum carougeaui, Stysanus stemonitis, Trichoderma viride, Ustilago zeae.*

CONCLUSION AND SUMMARY

Dermatophytes attack the hair in vitro; two methods of attack have been described. The dermatophytes are apparently the sole fungi which can attack the hairs in this manner. It is possible from the study of this action to make a diagnosis of the dermatophytic nature of the fungus. The observations made by the author prove once more that the morphology of the dermatophytes in vitro is definitely different from in vivo even when the hair itself is the culture medium. The technic described by the author may be used in other fields of mycology or to study the morphology of fungi or to study the action of fungi on substances other than the hair (cotton, wool, fabrics and so on).

INSTITUT DE MÉDICINE TROPICALE PRINCE LÉOPOLD,
ANTWERP, BELGIUM

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THE NUTRITION OF PENICILLIUM DIGITATUM SACC.[1]

CHARLES L. FERGUS[2]

(WITH 10 FIGURES)

The production of physiologically active emanations by the common green mold of citrus fruits, *Penicillium digitatum* Sacc., which cause epinastic responses of the leaves of potato, tomato and sunflower (Miller *et al.*, **1940**), the "triple response" of etiolated pea seedlings and the acceleration of the ripening of green fruits (Biale and Shepherd, **1941**), has attracted considerable interest. These responses of test plant material are similar to those invoked by ethylene and it has been assumed that the emanation from the fungus is ethylene, although no chemical proof has as yet been offered to verify the assumption.

For an investigation of the physiology of ethylene production, *P. digitatum* appears to be the most promising plant material because of its ease of manipulation, the possibility of rigidly controlling environmental conditions and the rapidity of growth of the fungus. Before the physiology of ethylene production of *P. digitatum* could be studied, it was necessary to have information as to the carbon and nitrogen nutrition, growth factor requirements, etc., of the fungus in order that a synthetic culture medium of known chemical composition could be used.

Reports in the literature on various aspects of the nutritional relationships of *Penicillium digitatum* Sacc. are numerous, but most of these are incidental and were made during the course of

[1] Based on a portion of a thesis submitted to the graduate school of the Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Appreciation is hereby expressed by the author to Dr. J. W. Sinden for his advice and encouragement during the course of the work.

[2] Contribution No. 165 from the Department of Botany, Pennsylvania Agricultural Experiment Station. Authorized for publication August 20, 1951, as paper No. 1685 in the Journal Series.

an investigation not concerned specifically with determining the nutritional relationships of *P. digitatum*. The results of various workers are not in agreement, particularly in regard to the growth factor requirements and the suitability of different nitrogen compounds for growth of *P. digitatum*.

In summary, the following conclusions may be drawn from previous cultural studies: *P. digitatum* requires thiamin and will not grow unless it is added to the medium (Wooster and Cheldelin, **1945**); or *P. digitatum* requires the addition of extracts of natural materials or organic compounds to grow satisfactorily, although it will grow slightly in a synthetic medium without their addition (Thom, **1910**; Camp, **1923**; Marloth, **1931**; Raistrick *et al.*, **1931**); or *P. digitatum* will grow satisfactorily in a synthetic medium without added growth factors or extracts of natural materials (Woltje, **1918**; Chrzaszcz and Tiukow, **1930**; Kursanoff and Alexeyeva, **1938**; Pratt, **1944**). Where there are reports of failure of *P. digitatum* to grow satisfactorily in a synthetic medium with no added growth factors, the nitrogen source has been either sodium or potassium nitrate except for the report of Wooster and Cheldelin (**1945**).

A very important and significant contribution to the knowledge of the nutrition of *P. digitatum* was made by Pratt (**1944**). Using a synthetic medium with no growth factors added and with ammonium nitrate as the nitrogen source, growth was slow and slight. The addition of thiamin to the basal medium increased growth but not as much as when a trace element supplement was added. The increased growth was due mostly to the added zinc. The addition of zinc and yeast extract did not increase the amount of growth, but did markedly accelerate it. The addition of thiamin and hydrolyzed casein to the basal medium resulted in obtaining maximum mycelium production one day earlier than with yeast extract and in addition increased mycelium production slightly. Various amino acids with thiamin were also tested and improved growth over thiamin alone was observed with tyrosine and thiamin. Growth was benefited by other amino acids, but none gave increases equal to the growth on thiamin and hydrolyzed casein.

The results of various workers agree regarding the availability of different compounds as carbon sources for the growth of *P.*

TABLE I

GROWTH OF *P. digitatum* IN MEDIA CONTAINING VARIOUS NITROGEN SOURCES

Nitrogen source	Camp*	Marloth	Kursanoff and Alexeyeva	Wooster and Cheldelin**
$NaNO_2$		2 percent germination. No growth.		
KNO_3	50		Good	0
NH_4NO_3	80	30 percent germination. Good growth.	Trace	12
$(NH_4)_2SO_4$		30 percent germination. Good growth.	Trace	50
NH_4 tartrate				51
Urea				5
Glycine			Good	13
Aspartic acid				36
Peptone	100	30 percent germination. Good growth.	Best	32
Casein				67
Asparagin			Good	100
Asparagin plus casein				142
Asparagin plus peptone				72

* Percent growth based on 100 percent for growth in peptone.
** Percent growth based on 100 percent for growth in asparagin.

digitatum. Glucose, fructose, sucrose and galactose serve well as carbon sources (Kursanoff and Alexeyeva, **1938**, and Wooster and Cheldelin, **1945**). Maltose was found to be unsatisfactory as a carbon source (Wooster and Cheldelin, **1945**). Citric and malic acids supported appreciable growth (Wooster and Cheldelin, **1945**); whereas acetic, oxalic and tartaric acids supported little or no growth (Kursanoff and Alexeyeva **1938**, and Wooster and Cheldelin, **1945**).

No agreement is found in regard to the suitability of different compounds as nitrogen sources. TABLE I summarizes the results regarding growth in various nitrogen containing media.

In view of the discrepancies noted above, and as a preliminary to a contemplated study of the physiology of ethylene production of *P. digitatum*, the nutrition of this fungus was investigated.

MATERIALS AND METHODS

The organism used in this study was *Penicillium digitatum* Sacc. strain NRRL No. *1202*. Strain NRRL No. *786* and an isolate from grapefruit were also used in one experiment.

The basal culture medium [3] used had the following composition: sucrose 25.7 gm., NH_4NO_3 4.0 gm., KH_2PO_4 13.61 gm., $MgSO_4 \cdot 7H_2O$ 1.23 gm., $FeSO_4 \cdot 7H_2O$ 0.02 gm., 1.0 ml. of microelement solution (supplied the following final concentrations of the elements per liter: copper 0.02 mg., zinc 0.05 mg., molybdenum 0.01 mg., manganese 0.5 mg., and boron 0.5 mg.), and distilled water to make one liter. The distilled water used was redistilled in a pyrex glass distilling apparatus. The pH of this medium after autoclaving was 4.4 and was not changed since this is in the optimum pH range of *P. digitatum* (Kursanoff and Alexeyeva, **1938**; Marloth, **1931**; and Wooster and Cheldelin, **1945**). All chemicals used were of c.p. grade.

The culture vessels used were 250 ml. pyrex Erlenmeyer flasks which had been thoroughly cleaned in Alconox and hot water, rinsed three times in hot running water and finally rinsed in distilled water. Fifty ml. of medium were used per flask.

The inoculum was prepared by introducing sterile redistilled water into a tube slant culture of *P. digitatum* growing on potato-dextrose agar. The tube was then shaken and the spore suspension rapidly poured into an empty sterile test tube. By using a Levy counting slide the spore suspension was adjusted by dilution with sterile distilled water until one ml. contained 25,000 spores. One ml. of this suspension was pipetted into each 250 ml. flask. This is the same method of inoculation used by Wooster and Cheldelin (**1945**).

To determine whether the method of inoculation was supplying soluble growth factors from the potato-dextrose agar, an experiment was carried out in which the spores were collected with the usual technique, but were washed in sterile distilled water by centrifugation and decantation. The spores were washed three times and then adjusted to 25,000 spores per ml. In the basal medium

[3] Pratt's medium is the basal medium used in this investigation. His medium was chosen as the basal medium because (1) it is a purely synthetic medium and contains no natural compounds of unknown composition; (2) *P. digitatum* can grow well in the nutrients provided as shown by preliminary growth studies and also by Pratt's results; and (3) since Pratt (**1944**) used this medium, direct comparisons of the results obtained in this investigation can be made with his results.

with no added growth factors a similar growth curve to that of FIG. 1, curve A, was obtained, indicating that the method of inoculation was not supplying any growth factors.

The flasks were incubated in the dark at 25° C. ± 2°. The flasks were either grown in stationary culture, or were placed on a mechanical shaker after the spores had germinated, which required approximately eight hours. The mechanical shaker used was designed by Dr. G. H. Fleming, Dept. of Chemistry, Pennsylvania State College. A speed of eighty-eight cycles per minute was used with a swing of four inches.

At various time intervals, three flasks were removed from each series, the cotton plugs removed, and then the flasks steamed at 100° C. for five minutes. This shortened the time of filtering. After steaming, each culture was filtered through a tared Gooch crucible and washed three times with 5 to 10 ml. portions of hot distilled water to remove any residual sugar, minerals, or other material which might give an erroneous mycelial weight. The filtered washed mycelium was dried overnight in an electric oven at 70° C. The pH of the culture medium was determined at time of harvest (prior to steaming) by means of a Beckman glass electrode pH meter.

Each value reported is the average of three flasks. Most of the experiments were set up so that values over an extended period of time could be taken. Previous investigators had shown that a single sampling may lead to erroneous results depending upon whether growth is at a maximum, or whether autolysis has commenced with subsequent weight loss.

EXPERIMENTAL RESULTS AND DISCUSSION

A. The Effect of Mechanical Shaking.

Kluyver and Perquin (**1933**) clearly demonstrated the advantages of mechanical shaking for producing in relatively short periods of time large amounts of cell material with high metabolic activity. Preliminary studies showed that replicate values obtained by growing *P. digitatum* on the mechanical shaker were more comparable than those obtained from stationary cultures since they did not vary as much as values obtained from stationary cultures. In

addition, maximum growth was obtained earlier than in unshaken flasks. The fungus grew as small round pellets on the shaker, with a minimum of sporulation, making the harvesting of mycelium much easier than from stationary cultures. Therefore, in most of these studies the fungus was grown on the shaker.

FIG. 1, curve A depicts the growth of *P. digitatum* in the basal medium in shaken flasks. Initiation of growth was earlier, and in addition the growth rate was accelerated slightly. Since there was less lag period in the early stages, the maximum growth was obtained two or three days earlier than in the stationary flasks, FIG. 1, curve B. The pH changes invoked in the basal medium as a result of the metabolic activity of *P. digitatum* were followed, and these changes are shown for both the stationary flasks, FIG. 1, curve C, and the shaken cultures, curve D. The initial pH drop due to shaken growth of *P. digitatum* was more rapid than that due to the unshaken growth. As growth proceeded, the pH in both series dropped to about 2.2. In the stationary series the pH remained slightly higher than in the shaken series. There was no autolysis of mycelium in the shaken flasks, whereas some autolysis did occur in the stationary series. The drop in pH is probably explained by the production of an acid or acids by *P. digitatum*, and also by the lack of utilization of the nitrate ion of ammonium nitrate resulting in the more acid condition.

B. The Utilization of Different Nitrogen Compounds.

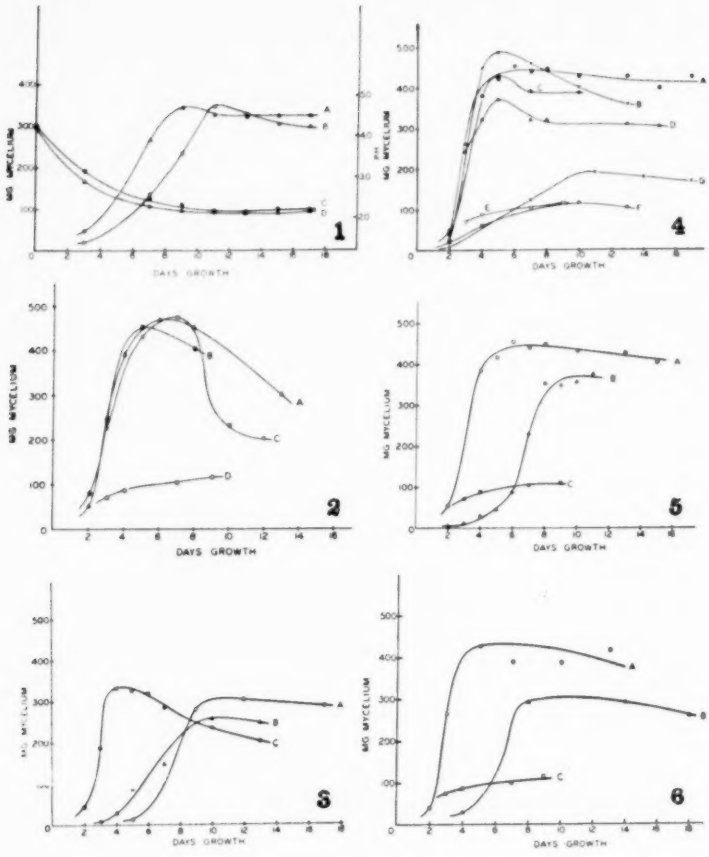
A review of previous work on the utilization of different nitrogen compounds by *P. digitatum* revealed little or no agreement as to which compounds favored best growth. The following series of experiments were performed with the purpose of discovering which compounds were most favorable for growth of the fungus in a synthetic medium of known chemical composition.

Ammonium nitrate was omitted from the basal medium and various organic and inorganic nitrogen compounds were substituted singly in amounts to give 1.4 grams of nitrogen per liter. Peptone was considered to be 16 per cent nitrogen. One gram of yeast extract was added to each liter of the basal medium. In addition, one series received no nitrogen other than that which is added in 1 gram of yeast extract. The results of this experiment are pre-

sented in FIG. 2. The growth of *P. digitatum* in peptone, asparagin and urea was quite similar, and the maxima obtained do not appear to be significantly different. The pH changes invoked in the media were observed. When urea was the nitrogen source, FIG. 2, curve C, the growth of *P. digitatum* caused the pH to drop from the original 5.8 to 3.9 by the third day. There was a gradual rise to 6.1 by the seventh day, and at the twelfth day a value of 5.0 was recorded. With asparagin as the nitrogen source, FIG. 2, curve B, growth of the fungus caused a slight drop from the original 4.4 to 3.6, followed by a rise to 5.3 by the eighth day. With peptone as the nitrogen source, FIG. 2, curve A, there was an original drop from the initial 5.1, to 3.3 by the fourth day, and then this was followed by a rise to 5.3 by the thirteenth day.

FIG. 3 represents the growth of *P. digitatum* in media containing the same three organic nitrogen compounds, but with no added growth factors. When peptone served as the nitrogen source, curve C, the rate of growth was approximately the same as the rate when the medium contained yeast extract (see FIG. 2, curve A), but the maximum mycelial weight produced was less. If the maximum mycelial weight produced on yeast extract alone is added to the maximum mycelial weight obtained with peptone alone, the total maximum weight is approximately the same. The initial growth was also practically the same. These favorable results obtained with peptone are probably due to the presence of various water-soluble vitamins in the peptone (Stokes *et al.*, **1944**). The pH changes were very similar to those reported when yeast extract was present.

When asparagin was substituted in the basal medium (FIG. 3, curve B) for the ammonium nitrate with no added yeast extract, the initial growth was not as rapid as when the yeast extract was present, nor as rapid as when peptone was present. In addition, the maximum mycelium produced was less. The pH changes in this medium as a result of growth were very similar to those reported before when yeast extract was added. However, autolysis was not very rapid nor marked. Fig. 3, curve A shows the growth of *P. digitatum* when urea was substituted in the basal medium. Here again, the initiation of growth was delayed, more time being required for the fungus to establish itself. Once growth was in-



FIGS. 1–6.

FIG. 1. Growth, B, and pH changes, C, in the basal medium in stationary flasks. Growth, A, and pH changes, D, in the basal medium in shaken flasks.

FIG. 2. Growth in basal medium with 0.1 percent yeast extract with various organic nitrogen sources. A peptone, B asparagin, C urea, and D yeast extract alone. Shaken.

FIG. 3. Growth in basal medium with different organic nitrogen sources. A urea, B asparagin and C peptone. Shaken.

FIG. 4. Growth in basal medium with 0.1 percent yeast extract with various inorganic nitrogen sources. A ammonium nitrate, B ammonium monohydrogen phosphate, C ammonium chloride, D ammonium sulphate, E yeast extract alone, F sodium nitrate, and G potassium nitrate. Shaken.

itiated the rate of growth was almost the same as that when peptone was the nitrogen source.

If the maximum mycelial weight produced in yeast extract alone (FIG. 2, curve D) is added to the maximum mycelium produced in asparagin alone or urea alone, the totals do not equal the maximum mycelium obtained when the yeast extract is added to the media containing these two compounds as nitrogen sources (see FIG. 2, curves B and C). The explanation of this is not known.

A similar series of experiments was conducted in which different inorganic nitrogenous compounds were tested in the basal medium with added yeast extract. FIG. 4 depicts growth of *P. digitatum* in the basal medium with various inorganic nitrogen sources with one gram of yeast extract added per liter of medium. When the nitrogen source was an ammonium salt, *P. digitatum* grew well, but when a nitrate (with the exception of ammonium nitrate) was the nitrogen source, the fungus grew poorly. When sodium nitrate (FIG. 4, curve F) was the nitrogen source no more growth occurred than in the yeast extract control. Nor was potassium nitrate a good source of nitrogen for this fungus (FIG. 4, curve G); however, it was better than sodium nitrate since the maximum mycelial production was 63 percent greater than in yeast extract alone, FIG. 4, curve E.

For the study of inorganic nitrogen compounds in a medium lacking any added growth factors two ammonium compounds and two nitrate compounds were tested. FIG. 5 depicts growth in ammonium nitrate with and without added yeast extract. FIG. 6 represents growth with ammonium chloride as the nitrogen source without added yeast extract. Both of these ammonium compounds proved to be satisfactory for growth of *P. digitatum*. The utilization of potassium nitrate and sodium nitrate was investigated in a similar manner, but because of the small amount of growth observed, the results are not presented as a figure. With potassium

FIG. 5. Growth with ammonium nitrate. A with 0.1 percent yeast extract added, B with no yeast extract, and C 0.1 percent yeast extract as the only nitrogen source. Shaken.

FIG. 6. Growth with ammonium chloride. A with 0.1 percent yeast extract added, B with no yeast extract, and C 0.1 percent yeast extract as the only nitrogen source. Shaken.

nitrate as the nitrogen source, growth was slow, requiring 40 days to attain the maximum dry weight of 90 mg. If this 90 mg. is added to the maximum obtained from yeast extract alone, 119 mg., the total weight is 209 mg. This is approximately the same as the mycelium produced in a medium containing both potassium nitrate and yeast extract (see FIG. 4, curve G). However, a much longer period of time was required to attain this amount of mycelium. With sodium nitrate as the nitrogen source with no added growth factors in the medium the fungus failed to grow.

In summary it may be stated that ammonium compounds or compounds such as peptone and urea which yield ammonia are excellent nitrogen sources, whereas nitrate compounds are poor sources of nitrogen. In the basal medium the production of a very low pH (see FIG. 1, curves C and D) strongly suggests that the nitrate ion of the ammonium nitrate is not being absorbed by the fungus, resulting in the more acid condition. These results also indicate that accessory growth factors are not necessary for the growth of *P. digitatum*, but if they are added, both the initiation of growth and the rate of growth are accelerated.

C. The Utilization of Different Carbon Compounds.

As has been pointed out earlier, the previous workers agree on the merits of various carbon compounds for the growth of *P. digitatum*, but only a few compounds have been investigated. In an experiment performed to obtain information as to the ability of *P. digitatum* to produce ethylene in a wide variety of organic media, information as to the ability to utilize different organic compounds as carbon sources was also obtained. Since the mycelial weights were obtained at only one sampling date, rarely two, none of these results can be considered as final in any sense, since an experiment which accounts only for growth at one fixed period is not absolutely comparative. This is readily seen by reference to any of the time-growth curves obtained in this study. The value obtained would be dependent, not alone upon the ability of the fungus to utilize the compound for growth, but also on whether germination is slow in the particular medium, or whether autolysis has commenced with subsequent weight loss.

If these restrictions are kept in mind the results presented in

TABLE II do yield some information as to the carbon nutrition of *P. digitatum*. The experimental procedure was modified as follows: 25 ml. portions of the basal medium in twice the normal concentration, without sucrose, were placed in 250 ml. Erlenmeyer flasks and autoclaved. Twenty-five ml. portions of the individual organic compounds were autoclaved in large Pyrex glass test tubes, and then were added to the 250 ml. Erlenmeyer flasks with sterile technique. The concentration of the carbon compound in the test tube was adjusted so that, upon dilution to 50 ml. in the Erlen-

TABLE II

GROWTH OF *P. digitatum* ON DIFFERENT CARBON SOURCES

Compound	Days growth	Milligrams of mycelium	Compound	Days growth	Milligrams of mycelium
D-Xylose	11	384	Dextrin	11	61
L-Arabinose	11	357	Dextrin	16	137
D-Glucose	11	377	Starch	16	9
D-Fructose	11	407	Citric Acid	11	106
D-Mannose	11	406	Fumaric Acid*	11	3
D-Galactose	11	372	Succinic Acid	11	9
L-Rhamnose	16	245	Oxalic Acid	11	0
D-Mannitol	11	66	Oxalic Acid	16	0
Sucrose	11	346	Lactic Acid	11	0
Sucrose	16	373	Lactic Acid	16	0
Maltose	11	79	Acetic Acid	11	0
Maltose	16	98	Acetic Acid	16	0
Lactose	11	242	L-Malic Acid	11	78
Lactose	16	339	Tartaric Acid	11	0
Cellobiose	11	381	Tartaric Acid	16	0
Inulin	11	2	Peptone	11	114
Inulin	16	2	Casein	11	133

* Only 0.35 gr. of fumaric acid was added to each flask because of its solubility.

meyer flask by the basal medium, each flask would contain 0.5410 gram of carbon. No growth factors were added. Soluble starch, dextrin and inulin were considered to be 44.4 percent carbon, and peptone and casein to be 52 percent carbon. The pH values of these media were found to be approximately 4.4 so no adjustment of pH was necessary. However, the pH values of the media containing the acids were very low, and consequently, adjustment to pH 4.4 was made using 30 percent potassium hydroxide. Each weight reported is the average of three replicate flasks.

The results show that when the two pentoses, D-xylose and L-

arabinose, are the carbon sources, *P. digitatum* grows well. A similar result was obtained with the hexoses, D-glucose, D-fructose, D-mannose, and D-galactose. L-Rhamnose yielded moderate growth, but when the polyhydric alcohol, D-mannitol, was present in the medium, only slight growth occurred. Of the disaccharides, sucrose and cellobiose yielded good growth, lactose moderate growth and only slight growth was recorded in maltose. Of the polysaccharides tested, inulin and starch could not be utilized, and when dextrin was present in the medium *P. digitatum* grew poorly. Malic and citric acids yielded the best growth of the acids tried, but the growth was not comparable to that obtained with the hexoses. Poor growth resulted in media containing succinic and fumaric acids, and no growth resulted in tartaric, lactic, oxalic and acetic acid media. Peptone and casein were not very satisfactory carbon sources.

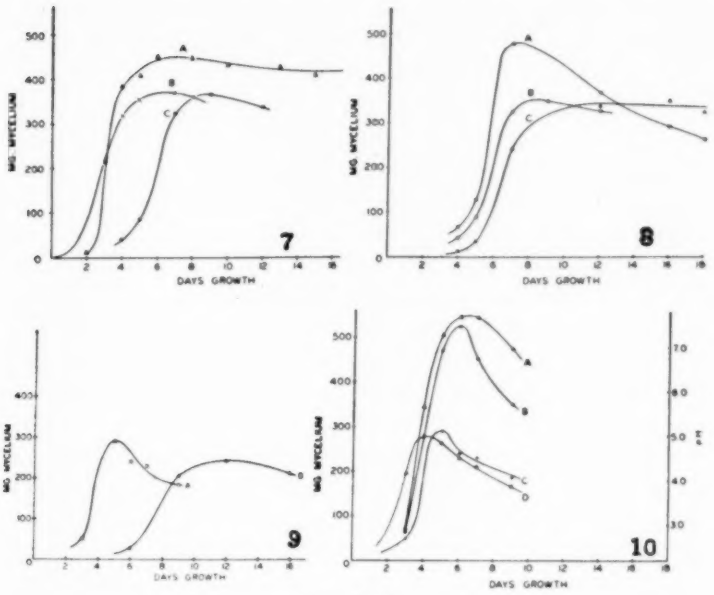
D. The Relation of Added Growth Factors to the Growth of *P. digitatum*.

As was pointed out in the introduction, contradictory reports have been made in regard to growth factor or vitamin requirements of *P. digitatum*. The following series of experiments was performed to determine whether or not this fungus can grow in a synthetic medium with no vitamins or growth factors present.

In summary of these experiments it may be stated that *P. digitatum* grows well in the basal medium with no added growth factors (see FIG. 7, curve C). However, the addition of 1 gram of yeast extract per liter, FIG. 7, curve A, results in a more rapid initiation of growth and a more rapid rate of growth. In addition, the maximum mycelial production was greater in this medium. This may be due to the extra organic nitrogen added so that more assimilation of protoplasm occurred.

A mixture of pure vitamins [4] was added to the basal medium. The growth of *P. digitatum* in this medium is presented in FIG. 7, curve B. The vitamin mixture allowed an earlier initiation of

[4] 400 micrograms of thiamin hydrochloride, 800 micrograms of pyridine hydrochloride, 600 micrograms of calcium pantothenate and 0.4 microgram of biotin per liter.



FIGS. 7–10.

FIG. 7. The effect of growth factors. A, basal medium with 0.1 percent yeast extract added, B, basal medium with vitamin mixture added, and C, basal medium with no added growth factors. Shaken.

FIG. 8. Growth of three different strains in the basal medium with no added growth factors. A, the grapefruit isolate, B, strain NRRL No. *1202*, and C, strain NRRL No. *786*. Shaken.

FIG. 9. Growth in unshaken flasks in Wooster and Cheldelin medium. A with usual vitamins present, and B with vitamins omitted.

FIG. 10. Growth in the Wooster and Cheldelin medium. Growth, C, and pH changes, B, in unshaken flasks. Growth, D, and pH changes, A, in shaken flasks.

growth than the basal medium alone (FIG. 7, curve C), but the maximum mycelium produced was practically the same.

These results agree with those of Pratt (**1944**), but are in direct contradiction to the results of Wooster and Cheldelin (**1945**). The vitamin mixture, and the vitamins, amino acids, purines and pyrimidines of the nucleic acids present in the yeast extract probably stimulate and accelerate initial growth before an optimum quantity has been synthesized by the fungus. The rate of growth (repre-

sented by the slope of the curve) was most rapid in the medium containing yeast extract. The rates of growth of the fungus in the basal medium and in the basal medium with vitamins added were the same. This would indicate that either a vitamin (or vitamins) other than the four used in the vitamin mixture, or some other stimulating substance (or substances) such as purines, pyrimidines or amino acids, is present in the yeast extract, resulting in the increased rate of growth.

Differences in the ability of different isolates of the same species to grow in media lacking any added vitamins have been observed by numerous workers. For example, Barnett and Lilly (**1947**) observed variations in the vitamin requirements among the isolates of the same species as well as among different species of Sclerotiniaceae.

Since *P. digitatum* strain NRRL No. *1202* can grow in the basal medium with no added growth factors in contradiction to the results obtained by Wooster and Cheldelin (**1945**), other strains were studied to determine if this strain is unique in its ability to grow without the addition of vitamins or growth factors. The other strains studied were NRRL strain No. *786*, and a strain isolated from a decaying grapefruit. Wooster and Cheldelin (**1945**) studied an isolate obtained from a decaying lemon. Attempts to obtain a culture of this isolate for comparative purposes failed.

The results of this study are presented in FIG. 8. From this experiment it may be concluded that the three strains studied grow well in the basal medium without any added growth factors. Each flask contained 1.285 gm. of sucrose or 0.5410 gm. of carbon. From this amount of carbon the grapefruit isolate produced 0.4824 gm. of mycelium, or a yield of 0.8917 gm. of mycelium per gram of carbon. Raistrick *et al.* (**1931**) had determined the carbon content of the mycelium of *P. digitatum* as 50 percent. If we assume that the mycelium of the grapefruit isolate is 50 percent carbon, 0.2412 gm. of the original 0.5410 gm. of carbon, or 44 percent, were utilized by this fungus in mycelial formation.

Stokes *et al.* (**1943**) observed that a mutant of *Neurospora sitophila* varied in its ability to grow without added pyridoxine, dependent upon the composition of the medium. Wooster and Cheldelin (**1945**) reported that *P. digitatum* will not grow unless

thiamin is added; however, their medium differs markedly from the basal medium used in this study. The following experiment was carried out to determine if the medium used by the above workers would support growth of *P. digitatum* strain NRRL No. *1202* if all vitamins were omitted.

The composition of the medium used by Wooster and Cheldelin (**1945**) was: KH_2PO_4 1.0 gm., $NaNO_3$ 1.0 gm., $MgSO_4$ 0.25 gm., KCl 0.10 gm., $CaCl_2$ 0.10 gm., $FeCl_3$ 5.0 mg., $MnCl_2$ 0.10 gm., $ZnCl_2$ 0.05 mg., H_3BO_3 0.05 mg., $CuCl_2$ 0.01 mg., KI 0.01 mg., sucrose 25.7 gm., asparagin 3 gm., ammonium tartrate 5 gm., casein hydrolysate (vitamin-free) 40 ml. 10 percent and a vitamin mixture of 400 micrograms thiamin hydrochloride, 800 micrograms pyridoxine hydrochloride, 600 micrograms calcium pantothenate and 0.40 microgram biotin and distilled water to make one liter.

The vitamins were omitted from this medium, and the growth of *P. digitatum* in unshaken flasks was studied. A control series containing the vitamins was also studied at the same time. The results are depicted in FIG. 9. A shaken series with vitamins added was also studied. The results are shown in FIG. 10.

The initiation of growth was more rapid, the growth-rate, once growth was initiated, was also more rapid, and the maximum mycelial production was greater in the medium containing the vitamins than in the medium lacking them. A rapid and extensive autolysis occurred in the medium containing the vitamins. The pH values were similar in the media with and without added vitamins, except that it required more time to attain the high pH of 7.4 in the medium lacking the vitamins than it did in the medium with vitamins.

Shaking accelerated the initial growth of *P. digitatum* in this medium, the maximum being obtained one day earlier than in the stationary flasks. Autolysis and pH changes in both of these series were similar.

Wooster and Cheldelin (**1945**) determined mycelial weights of unshaken cultures at the end of three days growth "because it permitted maximum growth before the onset of excessive autolysis." No results of growth over extended periods of time were reported, only the one sampling date being used. On the basis of the experiment depicted in FIG. 9, curve B, if mycelial weights had been

determined at the end of three days in the medium lacking vitamins, they would have been found to be slight; less than 10 mg.

SUMMARY

Cultivation of *P. digitatum* under the conditions of agitation and aeration on a mechanical shaker resulted in an acceleration of initial growth. In addition, replicate values obtained were more comparable than corresponding values obtained in stationary cultures, since they did not vary as much.

Peptone, urea and asparagin, both with and without added yeast extract, in shaken flasks proved to be satisfactory nitrogen sources for the growth of *P. digitatum*. Inorganic ammonium compounds were likewise utilized as nitrogen sources for growth, both with and without added yeast extract; however, nitrates (with the exception of ammonium nitrate) were utilized poorly, or not at all.

On the basis of a single sampling date, or rarely two, *P. digitatum* can utilize D-xylose, L-arabinose, D-glucose, D-fructose, D-mannose, D-galactose, sucrose and cellobiose very efficiently as carbon sources; L-rhamnose and lactose allowed moderate growth; and D-mannitol, maltose and dextrin were utilized poorly. No growth occurred when inulin and soluble starch were the carbon sources. Citric and malic acids could be utilized to a certain extent, but fumaric, tartaric, lactic, acetic and oxalic acids could not be utilized.

P. digitatum strain NRRL No. *1202*, strain NRRL No. *786* and an isolate from grapefruit do not require the addition of growth substances to the basal medium for good growth. However, if yeast extract or a vitamin mixture of biotin, thiamin, pyridoxine, and calcium pantothenate are added to the basal medium, the initial growth of the fungus is accelerated.

DEPARTMENT OF BOTANY,
PENNSYLVANIA STATE COLLEGE,
STATE COLLEGE, PENNSYLVANIA

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THE INABILITY OF COPRINI TO SENSITIZE MAN TO ETHYL ALCOHOL

GEORGE P. CHILD

The recent introduction of tetraethylthiuramdisulfide "Antabuse" which sensitizes man to ethyl alcohol (**5**) has renewed the interest in other substances which presumably produce the same effect. A number of references appear in the literature which have ascribed this property to *Coprinus atramentarius* and *C. comatus* (**1**, **4**, **6**, **9**). It is alleged that if a small amount of alcohol is imbibed after eating these fungi, man becomes violently intoxicated, disoriented, and irrational for periods from 6 to 24 hours.

The detailed descriptions of some of these incidents indicate, however, that the intoxication appeared to develop even before the alcohol was imbibed (perhaps in anticipation). The signs and symptoms, moreover, appeared to resemble those seen after the ingestion of *Panaeolus* sp. (**3**, **7**, **8**). Because of the interest in obtaining suitable nontoxic drugs which could be used therapeutically in the treatment of chronic alcoholism this problem was investigated.

MATERIALS AND METHODS

C. atramentarius, *C. comatus* and *C. micaceus* were collected from a number of sites during the spring and autumn of 1950. They were washed and consumed raw or they were parboiled or fried lightly in butter. Each species was handled separately and all stages of development were represented. After its preparation the particular species was eaten in amounts ranging from 100 to 600 grams. The juices were also consumed.

During or after the meal an alcoholic beverage was consumed in an amount sufficient to indicate both subjectively and objectively whether alcohol sensitization had occurred. The same subject was used in all the tests. This subject had been selected for his ability to become sensitized to alcohol with other drugs and his reactions

were well known. The experiments were repeated at least 5 times for each species.

This individual also voluntarily ingested 2 grams of parboiled *Pancolus companulatus* in a separate test.

RESULTS

No untoward effects were noted after the ingestion of the *Coprini*, whether raw, fried or boiled. This genus also produced no sensitization to ethyl alcohol.

The small amount of *Pancolus*, however, produced a noticeable reaction without the use of alcohol. The subject's face became flushed, his eyes were glassy, the pulse rate increased and a slight dyspnea was experienced. These effects developed within 10 minutes after the mushroom was eaten, reached their maxima in 20–30 minutes and were practically gone in one hour with no sequellae.

DISCUSSION

Despite the limited nature of these experiments it seems that the effects ascribed to the Coprini could have resulted from the inclusion of *Pancolus* in the mushrooms which had been eaten. It would not be too difficult to mistake *C. atramentarius* or *C. micaceus* for *Pancolus*. These genera are often found near one another and a *Pancolus*, particularly *P. solidipes* with its dark lamellae, could easily be mistaken for a small *C. atramentarius* or *micaceus*. It is possible, however, that no mistake had been made and that Coprini collected from other sites or fed to other subjects might have alcohol-sensitizing effects (**2**).

SUMMARY

Coprinus atramentarius, *C. comatus* and *C. micaceus* were prepared in a number of ways and were eaten by a subject who was known to react to alcohol sensitizing drugs.

The Coprini produced no untoward effects and did not sensitize the subject to alcohol. *Pancolus*, however, produced a noticeable intoxication without the use of alcohol. It was suggested that the reports in the literature alleging that Coprini sensitize to alcohol

might be explained by the accidental inclusion of *Panaeolus* in the cooking vessel.

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY,
ALBANY MEDICAL COLLEGE,
ALBANY, NEW YORK

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PRELIMINARY OBSERVATIONS ON THE OCCURRENCE OF MITOSIS IN CARYOPHANON LATUM [1]

EDWARD D. DELAMATER [2]

(WITH 1 FIGURE)

INTRODUCTION

Peshkoff (**6**) isolated this organism from cow dung in 1940. Pringsheim and Robinow (**7**) re-isolated the organism in England from the same source. So far as the present author is aware, Peshkoff, and Pringsheim and Robinow, are the only workers who have studied this organism to date.

The nuclear structures were visualized by both authors, and were considered to be the long rod-like masses of chromatin lying transverse to the cell axis. Peshkoff felt that these divided longitudinally, and that the two halves subsequently separated. Robinow did not add further to this concept.

The purpose of this preliminary paper is to indicate the occurrence of a typical mitotic process in this organism.

MATERIALS AND METHODS

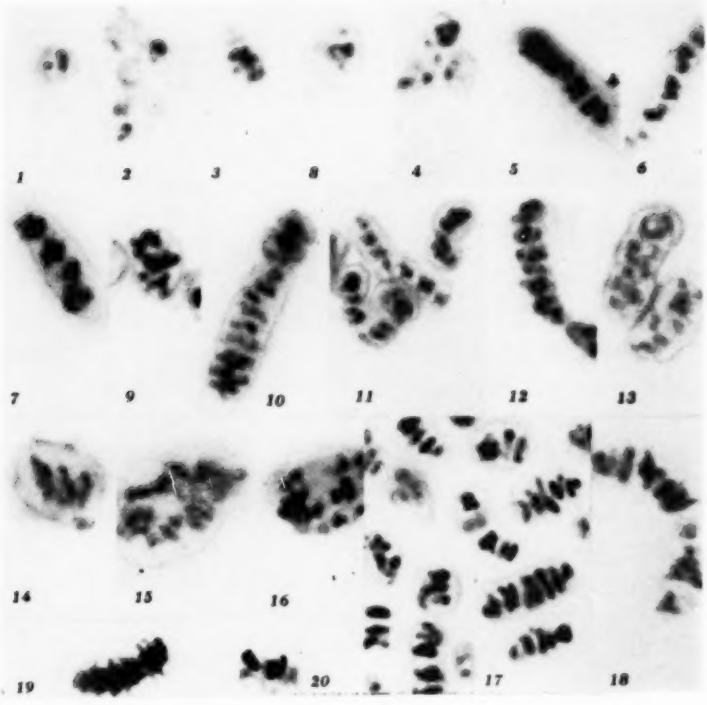
A culture of *Caryophanon latum* was kindly supplied by Dr. C. F. Robinow. It has been grown on yeast extract-rabbit dung agar. The cytologic procedures which have been used were according to the methods of DeLamater (**1**).

OBSERVATIONS

FIGS. 1, 2 and 3 demonstrate late prophase stages in which the chromosomes are contracted and lie opposite a single centriole. So far as it has yet been possible to ascertain, the centriole arises

[1] This study was supported in part by U. S. Army Grant No. W-49-007-MD-473.

[2] Research Professor of Dermatology and Syphilology, and Research Professor of Microbiology.



FIGS. 1–20.

FIGS. 1–20. Mitosis in *Caryophanum latum*. All figures × 3000. 1 and 2. Late prophase, showing clumped chromosomes opposite single centriole. 3. Slightly earlier prophase before chromosomes have become so tightly massed. 4 and 5. Later prophase, showing clumped chromosomes with divided centrioles migrating to poles of spindle. 6. Metaphase spindle with chromosomes on plate between two centrioles. 7 and 8. Anaphase, showing early separation of chromosomes. 9. Telophase. Clumps of chromosomes migrating toward persistent centrioles. 10. Early interphase. Nuclei condensed, being separated by cross wall. 11. Early interphase. Chromosomes separating. Central cell shows late interphase with chromosomes occurring as long, delicate threads. Nuclear membrane also evident. 12 and 13. Late interphase nuclei with chromosomes in long, tangled threads. 14. Early prophase in crushed cell. Two nuclei, each showing two chromosomes as thick, contracting threads. 15. Interphase in crushed cell. Chromosomes as long, delicate, beaded threads. 16. Interphase in crushed cell. Points of contact between chromosomes suggest formation of true spireme. 17 and 18. Late prophases, showing chromosomes as paired, thickening rods transverse to cell axis. 19. Rod, showing partial compartmentalization due to inward folding of cell membrane. Nuclei in late prophase. 20. Two nuclei, showing chromosomes as very short dense pairs of rods. Centriole not yet formed.

de novo at about this time. FIG. 3 probably represents a slightly earlier stage than FIGS. 1 and 2. FIG. 4 shows a much later prophase in which two centrioles have formed by division, and are migrating to the poles. FIG. 5 likewise demonstrates the separation of the centrioles. FIG. 6 shows a typical metaphase spindle lying transverse to the axis of the cell, and FIGS. 7 and 8 show anaphase stages in the separation of the chromosomes on the plate. The stage in FIG. 7 is somewhat later than that shown in FIG. 8. In FIG. 9 a typical telophase stage, in which the separating chromosomes are migrating towards the persistent centriole, is demonstrated. In FIG. 10 can be seen the late telophase or early interphase nuclei so formed, between which a transverse wall is being laid down. In FIG. 11 can be seen early interphase nuclei in which the chromosomes are beginning to separate one from another. In the central cell can be seen a late interphase nucleus in which the chromosomes appear in long threads, tangled within the nucleus. Similar stages can be seen in FIGS. 12 and 13. In FIG. 15 the long, beaded threads of the interphase nucleus are clearly visualized in a crushed cell, while in FIG. 16 a pair of chromosomes in the interphase is suggested. In FIG. 14, in a similarly crushed cell, the two chromosomes of very early prophase are seen to be contracting and becoming more dense. Later prophase contractions are shown in FIGS. 17 and 18. FIG. 17 is likewise noteworthy because it demonstrates the characteristic ladder-like pattern of the large vegetative cells described by both Peshkoff and Robinow. A late, well-condensed prophase, showing pairs of chromosomes, is shown in FIG. 20, while in FIG. 19 the compartmentalization of the cell, due to internal partial folding of the cell membrane with the production of perforate walls, is demonstrated.

DISCUSSION

The demonstration of mitosis in *Caryophanon latum* extends the observation of this nuclear process to a third bacterium, representing a third group of bacteria (**2–5**). It seems likely that the mitotic process is general among bacteria, and that it has been missed in the past only because of the lack of adequate methods. Further discussion in a definitive paper on this subject will be presented elsewhere.

A marked similarity between bacterial nuclei and the nuclei of certain fungi, particularly *Ascomycetes*, should be noted.

DEPARTMENTS OF DERMATOLOGY AND SYPHILOLOGY AND OF MICROBIOLOGY,
SCHOOL OF MEDICINE,
UNIVERSITY OF PENNSYLVANIA,
PHILADELPHIA, PENNSYLVANIA

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USTILAGO TURCOMANICA TRANZSCH. IN THE UNITED STATES [1]

GEORGE W. FISCHER AND JACK P. MEINERS

(WITH 1 FIGURE)

INTRODUCTION

In 1938 and again in 1939, a head smut was found on *Agropyron orientale* var. *lasianthum* (Boiss.) Boiss. in the observational row nurseries of the Soil Conservation Service, Pullman, Washington.[2] Although this grass is strictly exotic (introduced from Turkestan, Foreign Plant Introduction No. *119,605*) the smut was assumed to be *Ustilago bullata*, a common species causing head smut on many grasses and with the same general symptoms. It was reported in 1937, 1940, and 1945 (**2**, **4**, **5**) as *U. bullata*. In one of these reports (**4**) it was pointed out that the spore germination in this collection was not typical of *U. bullata* and that it might represent a different species. Subsequent studies have led to the conclusion that it represents *U. turcomanica* Tranzschel. It seems probable that the smut was introduced with the seed.

Tranzschel and Serebrianikov issued *Ustilago turcomanica* W. Tranzschel sp. n. on *Agropyron squarrosum* as Mycotheca Rossica No. *302*. Specimens of the type material were loaned from the Mycological Collections, U. S. Department of Agriculture, through the courtesy of J. A. Stevenson. The Washington State Collection on *A. orientale* var. *lasianthum* is morphologically identical with the type of *U. turcomanica*.

Gutner (**6**) gives *Agropyron orientale* as one of the hosts for *Ustilago turcomanica* and states that he has found this species only

[1] Scientific Paper No. 1048, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington.

[2] Collected by Carl Riesenweber. Cooperative investigations of the Washington Agricultural Experiment Stations; Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering; and the Soil Conservation Service, Section of Nurseries, United States Department of Agriculture.

in the Soviet Union.[3] Although this smut apparently was introduced into the United States with seed of *A. orientale* var. *lasianthum*, there is very little possibility that it has become established. In calling attention to this introduction of a plant pathogen from Asia, it seems worth while to present the results of some studies of its biology.

PATHOGENICITY STUDIES

In 1939, seeds of the following grasses were inoculated with a spore suspension of *Ustilago turcomanica* by the partial vacuum method: *Agropyron trachycaulum* (Link) Malte, *Bromus brizaeformis* Fisch. and Mey., *B. carinatus* Hook. and Arn., *B. catharticus* Vahl, *B. erectus* Huds., *B. hordeaceus* L., *B. inermis* Leyss., *B. japonicus* Thunb., *B. mollis* L., *B. rubens* L., *B. secalinus* L., *B. squarrosus* L., *B. tectorum* L., *Elymus canadensis* L., *E. glaucus* Buckl., *E. sibiricus* L., and *Hordeum nodosum* L. These grasses comprised the differential hosts used at that time in physiologic race studies of *U. bullata*. Only *E. glaucus* became infected, with smut in approximately 20 percent of the heads.

In 1940, the same grasses, plus *Agropyron inerme* (Scribn. and Sm.) Rydb., were inoculated. This time approximately 50 percent smut appeared in *Elymus glaucus*, 40 percent in *E. sibiricus*, 10 per cent in *A. inerme*, and five percent in *E. canadensis*.

In 1947, the following grasses were inoculated with *Ustilago turcomanica*: *Bromus carinatus*, *B. catharticus*, *B. hordeaceus*, *B. japonicus*, *B. mollis*, *B. tectorum*, *Elymus canadensis*, *E. glaucus*, *E. sibiricus*, and *Hordeum nodosum*. Twenty-seven percent smut appeared in *E. canadensis*, 17 percent in *E. glaucus*, and one percent in *E. sibiricus*.

The results of these inoculation experiments show that species of *Elymus* and *Agropyron* are hosts of *Ustilago turcomanica* and that infection occurs in the seedling stage.

SPORE GERMINATION

Brefeld (1) described spore germination in *Ustilago turcomanica* from material Hennings had sent him from Turkestan on *Agro-*

[3] The authors are indebted to Austin Goheen for translation from the Russian.

pyron orientale but which Brefeld identified as *U. bullata*. He described germination in water and nutrient solution. In water one to several germ tubes emerged from a single spore. Brefeld's illustrations clearly indicate that some of the "germ tubes" were in reality infection hyphae.

Some observations were made on spore germination in the present studies of *Ustilago turcomanica*. Spores up to seven years old germinate readily, although the percentage germination is lower in spores three or four years or more of age. Spores sown on potato dextrose agar and incubated at room temperature commence to germinate in 24 to 36 hours. Two to four short, thick, germ tubes emerge from a rift in the spore wall (FIG. 1, A–D). These germ tubes are septate and probably correspond to the promycelia of *U. bullata*, and other species in which the promycelial cells produce successive crops of sporidia. That these germ tubes are really promycelia is evidenced by the frequent occurrence of "knee-joints," which result from sexual reaction between adjacent cells. This process consists in the formation of a protuberance at the cross wall and eventual protoplasmic continuity between the two adjacent cells (FIG. 1, A, a), which presumably permits pairing of the nuclei. The protuberance elongates (FIG. 1, B, a; C, a) and develops an infection hypha.

One of the most characteristic features of the spore germination in *Ustilago turcomanica* is the development on the promycelium, or its branches, of sporidium-like bodies (FIG. 1, C, b and c; D, a and b). These resemble sporidia produced by other smut fungi in the Ustilaginaceae but differ by the fact that they are detached only with difficulty. Some of these detached sporidium-like bodies are shown in FIG. 1, D.

BEHAVIOR IN CULTURE

Although detached typical sporidia are seldom found, sporidium-like branches are produced which can be isolated and which will bud and develop into colonies composed of a tough mycelium and more "sporidia." Using a Chambers micromanipulator, 18 mono-"sporidial" cultures were obtained—five from each of two germinating spores and eight from a third. With no definite single promy-

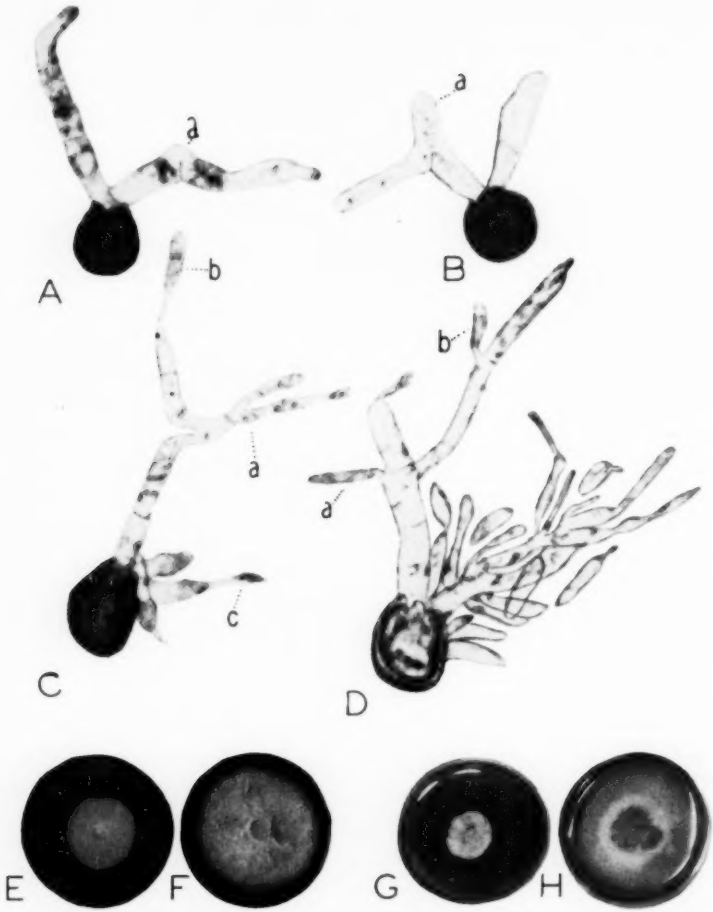

FIG. 1. *Ustilago turcomanica*. A–D. Photomicrographs of spores germinated on potato dextrose agar and stained *in situ* with cotton blue, somewhat retouched. A, a and B, a. Areas of protoplasmic continuity between adjacent cells of opposite sex; B, a, shows the beginning of elongation of one of these areas to develop into an infection hypha; C, a and c; D, a and b. Sporidium-like cells; D, advanced stage of germination showing some detached sporidium-like cells. E–H. Four mono-"sporidial" cultures after four weeks on 50 mm. petri plates of potato dextrose agar; E and F are from the same chlamydospore but are of opposite "sex" or compatibility groups; G and H, *ibid.* but from a different spore. In both pairs, note the sex-linked differences in character and rate of growth. A–D, × approx. 1030; E–H, × approx. 9/20.

celium developing from a spore, it was impracticable to try to "pedigree" the cultures with reference to their origin from different cells of the promycelium.

Ustilago turcomanica grows readily on artificial media. The 15 cultures were easily maintained on test tube slants of potato dextrose agar with two percent dehydrated malt extract broth added. Some of the cultures were grown in petri plates in order to better observe cultural behavior. Quadruplicate plates (10 × 50 mm.) were inoculated and incubated at room temperature for four weeks. FIG. 1, E–H, shows the appearance at the end of this time of two cultures of opposite sex from each of two spores. It is readily seen that there are sex-linked differences in rate and type of growth. N-R *71* and *75*, shown in FIG. 1, E and F, respectively, are both from the same chlamydospore and represent different sex or compatibility groups, as described later. Culture N-R *75* almost filled the diameter of the dish, while N-R *71* grew only a fraction as much. The same behavior characterized N-R *81* and *82* FIG. 1, G and H, respectively. This sex-linked difference in growth type and rate is further substantiated by the fact that N-R *82* and *75*, although from different spores, are similar in growth type and rate and are of the same sex or compatibility group. The same relationship holds for N-R *81* and *71*. All cultures were mycelial in consistency, but the slow-growing cultures were more raised and fluffy in contrast to the flat, fimbriate type of growth characterizing the fast-growing cultures.

SEXUALITY

Evidence of sexual manifestations was seen in the promycelia, as already described, where fusions frequently occurred between adjacent cells and gave rise to infection hyphae. Brefeld (**1**) described and illustrated these in *Ustilago turcomanica*, but apparently without knowing what they were.

As a further test of sexual manifestations in *Ustilago turcomanica*, 18-mono-"sporidial" cultures, representing 3 germinating spores, were mated in all combinations on three percent water agar. The results of these pairings indicated two sex or compatibility groups. Pairing of cultures of opposite sex was evidenced by an abundance of fusions and infection hyphae.

SUMMARY

The probable introduction into the United States of *Ustilago turcomanica* on *Agropyron orientale* var. *lasianthum* from Turkestan is described. This smut fungus is somewhat similar to *U. bullata* but is considered distinct from that species by characteristics of spore germination. Inoculations have shown that species of *Elymus* and *Agropyron* are hosts and that infection occurs in the seedling stage of the host.

Characteristics of spore germination, behavior in culture, and sexuality are described.

DEPARTMENT OF PLANT PATHOLOGY,
THE STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON

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LEAF SPOT OF PARSNIP CAUSED BY PHLEOSPORA CRESCENTIUM (BARTH.) N. COMB.

E. A. Riley [1]

(with 2 figures)

Leaves of parsnip (*Pastinaca sativa* L.) covered by minute brown to black spots were received at this laboratory during July, 1950. Examination of the spots showed that these were covered with crescent-shaped spores borne in what appeared to be acervuli. The

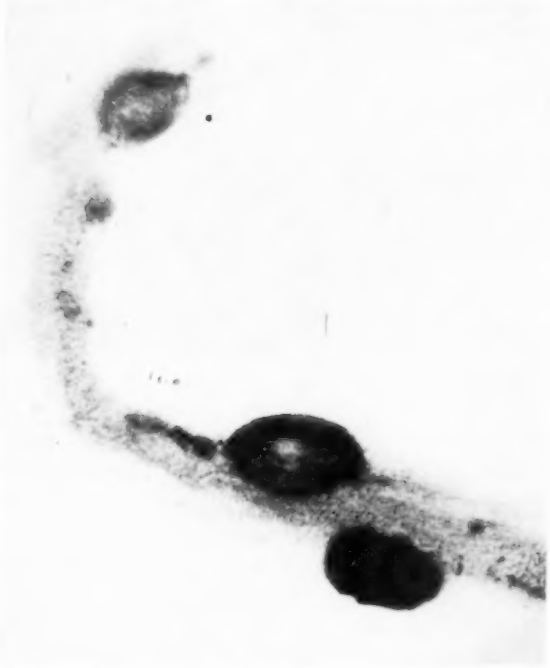

Fig. 1. Immature pycnidia embedded in the leaf, × 100.

[1] Plant Pathologist, Department of Research and Specialist Services, Southern Rhodesia.

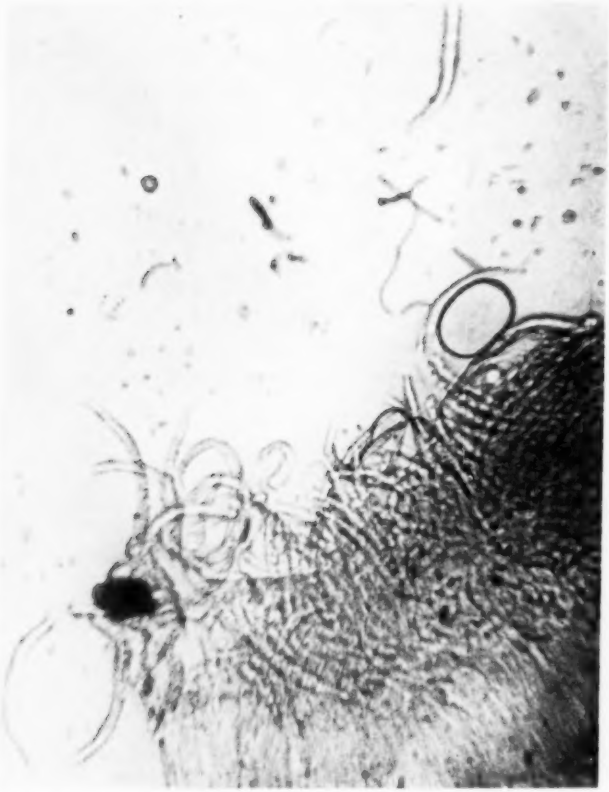

FIG. 2. "Acervulate" stage showing typical crescentiform spores, × 440.

fungus was provisionally assigned to *Cylindrosporium* and sent to the Commonwealth Mycological Institute for further identification. A subsequent report from the C.M.I. stated that the fungus was identical with *Cylindrosporium crescentum* Barth., of which authentic material was available, but that the genus was correctly *Phleospora*, the spores being produced in pycnidia which open at maturity to form "acervuli."

Bartholomew's description (Fungi Columbiani, No. *3617*) is as follows: "Spots minute, ochraceous, evenly scattered, becoming confluent over large areas which, in drying, turn brown. Acervuli amphigenous, innate, erumpent, light brown, turning black. Co-

nidia acuminate, subclavate, obtuse to acute, crescentiform, multinucleate, continuous, hyaline, 45–80 × 3–5 μ. With age the host becomes blotched with white specks from the profusely discharged conidia. On leaves of escaped *Pastinaca sativa*."

On the basis of the foregoing remarks, I propose that the fungus be transferred to *Phleospora,* the description being amended as below:

Phleospora crescentium (Barth.) n. comb., syn. *Cylindrosporium crescentium Barth.* as *C. crescentum,* Fungi Columbiani, No. *3617.*

"Pycnidia scattered or aggregated into clusters, innate, erumpent, subglobose, dark brown, at first covered, then open, 281 (190–380) μ at maturity. Conidia acuminate, acute to obtuse, crescentiform, hyaline, 1-septate (few continuous), granular, 67.9 (38–83) × 4 (3–6 μ). Conidiophores cylindrical, hyaline, continuous, 19–26 × 2–4 μ. Causing leaf spot of cultivated *Pastinaca sativa*."

Bartholomew described the spores as continuous, but in the C.M.I. preparation of authentic material they were nearly all 1-septate.

THE ASCIGEROUS STAGE OF SEPTORIA QUERCETI THUEM.[1]

JOSEPH C. GILMAN AND BRYCE N. WADLEY

(WITH 1 FIGURE)

The leaf-spot of oak, particularly that of the red oak group, occurs frequently throughout Iowa. The spots are circular in outline, about 2–3 mm. in diameter, with a straw-colored center and a dark brown margin, scattered rather generally over the infected leaf (FIG. 1, A). In the pale centers the black ostioles of the pycnidia of *Septoria querceti* Thuem. protrude (FIG. 1, B). An infected tree on the campus of Iowa State College gave opportunity to observe the development of the disease intensively. Although the leaves were almost uniformly spotted as shown in the illustration, defoliation did not occur, but the growth of the infected tree was retarded when compared with that of an uninfected tree of the same species near by.

In 1941, examination of overwintered leaves from the infected tree disclosed the presence of perithecia containing asci and ascospores of a *Mycosphaerella*, but the investigation was interrupted until 1945. In that year, infected leaves were collected in November and placed on the soil outside the greenhouse under a greenhouse flat for protection. These leaves were examined at intervals in March of the following year and late in the month perithecia similar to those observed in 1941 were found containing mature ascospores. Attempts were made to isolate the fungus from this material at that time but without success. Collections were repeated in the fall and in April 1947 the mature perithecia again were obtained. By fastening washed pieces of those leaves showing perithecia to the covers of petri dishes over 2% nonnutrient agar, discharged ascospores were caught and single asco-

[1] Journal Paper No. J-2008. Dept. of Botany and Plant Pathology, Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 110.

spores were transferred to test tubes of potato dextrose agar. The resulting cultures produced pycnidia and pycnidiospores similar to those produced by isolates from the conidial producing spots that earlier had been identified as *Septoria querceti*. Although no ascigerous stage has been produced in our cultures we feel reasonably sure that the *Mycosphaerella* is connected with the *Septoria* as being part of its life cycle.

The perithecia on the overwintered leaves were globose with a short papillate ostiole (FIG. 1, E), 156–208 μ (182.6 μ) in diameter. The asci (FIG. 1, F) were 60–70 × 8–10 μ (68.2 × 8.75) and the ascospores (FIG. 1, G) were 25–29 × 3.5–4 μ (27.5 × 3.6). The conidia (FIG. 1, D) were 17–35 × 2.5–4 μ (25.4 × 3.5) contained in globose pycnidia (FIG. 1, C) that were slightly larger than the perithecia.

Weiss (**4**) in his check list has brought together the species of *Mycosphaerella* described from different species of oaks, and Miller (**3**) has listed the Georgia species on this same group of hosts. These species are summarized with their ascospore dimensions in TABLE I. *Mycosphaerella catesbeyi* (Cke.) Miller is the only species whose spores approach the length of our material. Examination of material of *M. catesbeyi* in the Herbarium of the United States Department of Agriculture showed this species to be distinctly different from that from Iowa. The leaves did not show the characteristic spotting caused by our fungus and the perithecia were scattered over large areas of the leaf, not confined to local spots. These characteristics together with the longer spores of the Iowa fungus have led us to consider it an undescribed fungus with the following characteristics:

Perithecia numerous, hypophyllous on circular straw-colored spots with brown margins, black, globose with erumpent papillate ostioles, 156–208 (182.6) μ in diameter. Asci fasciculate, cylindric, aparaphysate, 60–70 × 8–10 (68.2 × 8.75) μ; ascospores inordinately biseriate, elongate, uniseptate, hyaline, 25–29 × 3.5–4 (27.5 × 3.6) μ. Conidial stage: *Septoria querceti* Thuem. Pycnidia in pale circular spots with a narrow brown margin, hypophyllous, numerous, innate then erumpent by a papillate ostiole; conidia scolecoform, curved, uniseptate, 17–35 × 2.5–4 (25.4 × 3.5) μ.

On overwintered leaves of *Quercus* spp. (cult.), Ames, Iowa.

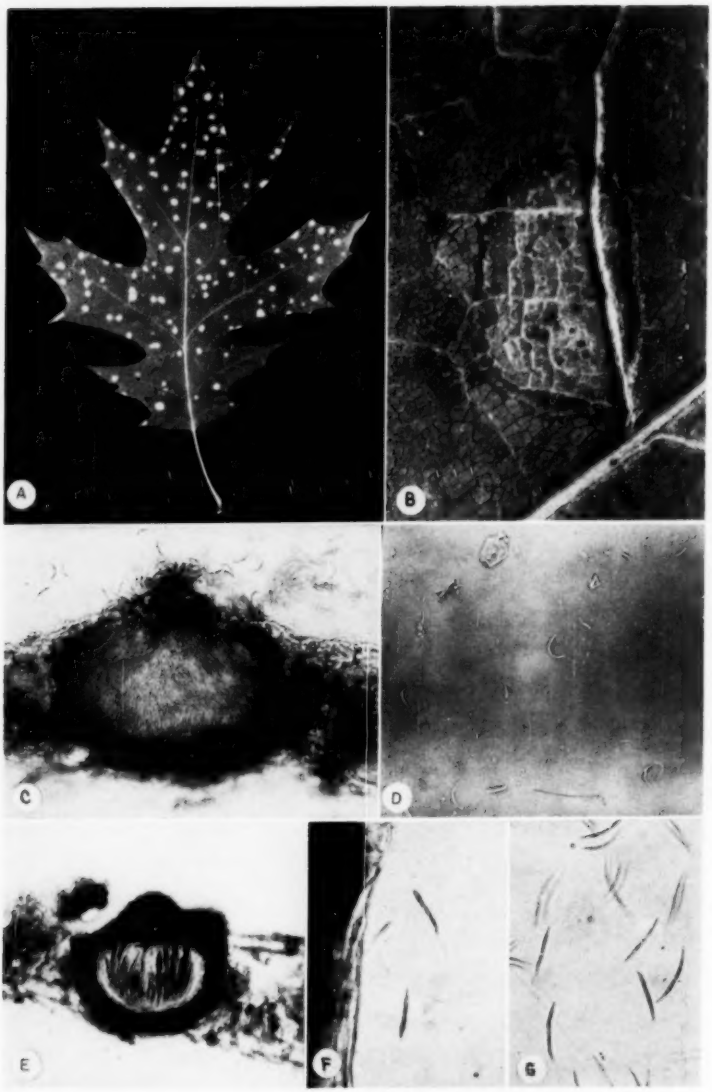

FIG. 1. *Mycosphaerella psilospora* on oak. A. Typical spots caused by the fungus, × ½. B. Typical spot enlarged × 20 showing mouths of pycnidia. C. Section through pycnidium, × 100. D. Conidia, × 100. E. Section through perithecium, × 100. F. Asci, × 100. G. Ascospores, × 350.

TABLE I

SPECIES OF *Mycosphaerella* REPORTED FROM OAK IN THE UNITED STATES

Species	Hosts	Ascospore dimensions
M. caespitosa Ell. & Ev.	*Quercus lobata* Nee *Quercus virginiana* Mill.	8–10×2.5–3 μ
M. punctiformis (Pers. ex Fr.) Schroeter	*Quercus alba* L.* *Quercus marilandica* Muench.* *Quercus maxima* (Marsh) Ashe* *Quercus montana* Willd.* *Quercus nigra* L.* *Quercus phellos* L.* *Quercus prinus* L.* *Quercus stellata* Waugh.* *Quercus velutina* L.*	6–9×2–3.5 μ
M. spleniata (Cke. & Pk.) House	*Quercus alba* L.* *Quercus austrina* Small* *Quercus lobata* Nee *Quercus macrocarpa* Michx. *Quercus margaretta* Ashe* *Quercus prinus* L.* *Quercus stellata* Wang.*	12–15×3–4 μ
M. operculata Sacc.	*Quercus chrysolepsis* Lieb.	13–16×3–4 μ
M. maculiformis (Pers. ex Fr.) Schroet.	*Quercus coccinea* Muench * *Quercus maxima* (Marsh) Ashe* *Quercus prinus* L.	14×3–4 μ
M. nigrita (Cke.) Miller	*Quercus lyrata* Walt.* *Quercus macrocarpa* Michx.	15×4 μ
M. aquatica (Cke.) Miller	*Quercus nigra* L.* *Quercus virginiana* Mill.	20×4 μ
M. catesbeyi (Cke.) Miller	*Quercus velutina* Lam. *Quercus catesbaei* Michx.	20–22×4 μ

* Reported by both Miller (**3**) and Weiss (**4**).

Mycosphaerella psilospora n. sp.

Peritheciis hypophyllis, globosis, atro-brunneis, in maculis orbicularibus, primo cuticula tectis, demum emergentibus; ascis clavato-cylindricis, 60–70 × 8–10 μ; sporidiis elongatis, uniseptatis, 25–29 × 3.5–4 μ.

Status conidiiferus: pycnidiis hypophyllis, numerosissimis, gregariis, minutis, immersis, punctiformibus; sporulis cylindraceis, suberectis v. arcuatulis, uniseptatis, multiguttulatis, hyalinis, 17–35 × 2.5–4 μ.

Hab. in foliis *Quercuum* spp.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
IOWA AGRICULTURAL EXPERIMENT STATION,
AMES, IOWA

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GNOMONIA FRAGARIAE IN MICHIGAN [1]

CONST. J. ALEXOPOULOS AND DONALD CATION

In a previous paper (**1**) we described a *Gnomonia* found associated with stem-end rot of strawberries in Michigan and stated that we believed it to be the perfect stage of *Dendrophoma obscurans*. A large number of isolations and comparative cultural studies [2] since then have convinced us that the fungus in question is *Gnomonia fragariae* Klebahn, an organism hitherto unreported from North America,[3] which is frequently associated with *Dendrophoma obscurans* but is distinct from it.

In our effort to identify our *Gnomonia* with some known species of this genus, we found *Gnomonia fragariae* Klebahn listed in the culture list of the Centraalbureau voor Schimmelcultures. After failing to find mention of this fungus in the standard taxonomic references (Saccardo, Oudemans, Seymour, etc.) the original description was found, through the courtesy of the Centraalbureau (letter dated February 6, 1948), to have been published in Klebahn's *Haupt- und Nebenfruchtformen der Askomyzeten* (**3**).

Klebahn discovered *Gnomonia fragariae* on strawberry petioles near Hamburg, Germany, in 1908. He described and figured the perithecial stage, but found no conidial stage on the host or in culture.

Nothing more appears to have been written concerning this fungus until 1931 when Professor and Mme. G. Arnaud (**2**) reported that they had found it in France the previous year, associated with a fruit rot of strawberries and with dead calyces and

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[2] The studies which have led to the conclusions reported herein were partially supported by a grant of the All-College Research Committee of Michigan State College, and grateful acknowledgment is hereby made to the Committee.

[3] After this paper had been accepted for publication, the presence of *Gnomonia fragariae* was reported in Ontario by Fall (Can. Jour. Bot. **29**: 299–315. 1951).

pedicels. Since they did not find the fungus on leaves and since Klebahn had not found it on fruits, Arnaud and Arnaud called their organism *Gnomonia fragariae* Klebahn forma *fructicola*. In addition to the ascigerous stage, Arnaud and Arnaud found a pycnidial stage associated with the perithecia on strawberry tissues and produced both stages in culture, noting that certain media favored the production of perithecia and other media favored the production of pycnidia. Since they did not work with monosporous cultures they did not actually prove the relationship of the two stages but they considered such relationship highly probable. It is of interest to note that although they described the asexual fruiting bodies as pycnidia they nevertheless referred to the conidial stage as *Gloeosporium ? fragariae*.

A comparison of our Michigan isolates with the description of *Gnomonia fragariae* given by Klebahn, and more especially with that given by Arnaud and Arnaud, shows that the Michigan fungus is very similar to the European organism. The similarity of our *Gnomonia* to the published descriptions of *Gnomonia fragariae* Klebahn prompted the senior author to send a culture (Michigan isolate *18AL 1a*) to Professor G. Arnaud early in 1950 for his valued opinion concerning the identity of the two forms. Upon examining our culture, which bore an abundance of perithecia and pycnidia, Professor Arnaud wrote (February 24, 1950) that in his opinion the fungus he had studied 20 years ago in France and our own discovered in Michigan in 1947 were the same species. *Gnomonia fragariae* Klebahn has also been found in Belgium by Van Holder in 1948 according to a communication from the Centraalbureau (April 6, 1950).

In 1944 Wormald (**4**) discussing leaf blotch of strawberries, a disease first found in England in 1941, stated that the causal organism is *Zythia fragariae* Laibach (*Phyllostricta grandimaculans* Bubak and Krieger). During his studies he discovered a *Gnomonia* perfect stage which he proved to be genetically connected with *Zythia fragariae*. Wormald did not name his *Gnomonia* but mentioned that it appeared to answer the description of *Gnomonia herbicola* on *Epilobium hirsutum*. He made no mention of *Gnomonia fragariae*. In our previous paper (**1**) referred to above,

we pointed out the similarity of our fungus to that of Wormald, but did not know of the work of Klebahn or Arnaud and Arnaud at that time. Unfortunately, cultures of *Zythia fragariae* received from England failed to sporulate and it has been impossible to compare the two organisms. It appears highly probable that the perfect stage of *Zythia fragariae* Laibach is *Gnomonia fragariae* Klebahn.

Since 1947 we have repeatedly found *Gnomonia fragariae* Klebahn, in its imperfect form, on leaves, pedicels, calyces, and fruits of strawberry in Michigan. Cultures made from single conidia have always yielded the typical perithecia. The perfect stage was found only once by us in nature. There is no explanation at present as to how this fungus known in Europe since 1908 suddenly appeared in Michigan 39 years later, but has not been found elsewhere on this continent. Since we have frequently isolated this fungus from typical *Dendrophoma obscurans* lesions on strawberry leaves it is our opinion that *Gnomonia fragariae* may be quite prevalent in this country and that it may have been overlooked in the field because of its frequent association with the well known and widely distributed *Dendrophoma obscurans*.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
MICHIGAN STATE COLLEGE,
EAST LANSING, MICHIGAN

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NEW SPECIES OF POLYPORES [1]

L. O. Overholts [2]

(WITH 1 FIGURE)

A manuscript copy of a manual on the Polyporaceae of the United States, Canada, and Alaska was found among Dr. Overholts' effects on his death. This manual has been prepared for publication by Josiah L. Lowe of the State University of New York, College of Forestry at Syracuse, and the manuscript is now in editorial processing at the University of Michigan Press at Ann Arbor. It was deemed desirable to publish separately the descriptions of the new species proposed by Dr. Overholts.

The descriptions have been prepared for publication by Dr. Lowe. The type material has been studied, some descriptive material added enclosed in brackets, and Latin diagnoses prepared.

Fomes repandus Overholts, sp. nov. Fig. 1.

Perennis, durus, effuso-reflexus vel resupinatus; pileus superficie ater, asper, induratus, 1–3 cm. reflexus, ad 4 cm. crassus, aliquando late effusus; contextus fulvus, ad 0.5 mm. crassus; superficies pororum fusca; tubuli indistinctis coriis; pori rotundati, 5–7 in uno mm.; sporae ellipsoidae vel ovales, leves, hyalinae, 3–4 × 2.5–3 μ; setae paucae, 7–10 μ in diametro, ad 20 μ eminentes; hyphae contextus septatae, 3–5 μ crassae. Specimen typicum in herbario Overholtsii conservatum, no. *11648*. Legit C. Epling in Upper Priest River, Idaho, July 1925.

Sporophore perennial, hard and woody, effused-reflexed or more usually nearly or quite resupinate and then broadly effused with a rusty-ochraceous margin that may become tumid, older part black, receding, never more than narrowly reflexed to a pileus length of 1–3 cm.; pileus with surface black, sulcate, and rough from the annual increments, not truly incrusted but the surface layer becom-

[1] Contribution No. 164 from the Department of Botany, Pennsylvania Agricultural Experiment Station. Authorized for publication July 6, 1951, as Paper No. 1679 in the Journal Series.

[2] Dr. Overholts died on Nov. 10, 1946. An account of his life and contributions to mycology appeared in Mycologia **40**: 1–5. 1948.

FIG. 1. *Fomes repandus*. Upper surface and vertical section views of the type collection. × 1.

ing blackish and hardened, glabrous, becoming as much as 3 cm. thick where best developed, the growing margin narrowly yellowish-brown; context less than 0.5 cm. thick, reddish-brown, rather soft to firm, fibrous; pore surface smoky-brown to dark brown, even, the tubes indefinitely stratified to the thicknesses of 1–2.5 cm., reddish-brown within, hard and woody, the pores circular, thick-walled at first, becoming rather thin-walled, entire, averaging 5–7 per mm.; spores ellipsoid or subglobose, smooth, hyaline, 3–5 μ in diameter; setae few, inconspicuous, short and blunt or at times broadly or narrowly conic, projecting up to about 20 μ, 7–10 μ in

diameter; [hyphae of the context rarely branched, thick-walled, inconspicuously septate, 3–5 μ in diameter].

HABITAT: On dead wood of coniferous trees; noted on *Larix*, *Pinus*, and *Pseudotsuga*.

DISTRIBUTION: Type collected at Upper Priest River, Idaho, July, 1925, by Dr. C. Epling (No. *1751*), Overholts Herb. No. *11648;* also from Priest River, Idaho (Weir Nos. *9547* and *8036*), Overholts Herb. Nos. *4491* and *3878;* from Roberts Creek, British Columbia, Irene Mounce (No. *3588*), Overholts Herb. No. *16592;* Cowichan Lake Forest Exp. Station, British Columbia, Overholts Herb. No. *24104;* and from Nordman, Idaho, Overholts Herb. No. *23820*.

Of the collections at hand, the type collection is the only one with well-developed pileate tendencies. The species differs from *Fomes nigrolimitatus* Rom. in the very different setae and spores as well as in other characters. Where resupinate specimens have been separated from the substratum, the sporophore surface is typically a bright yellow-red or rusty-red due to a thin spongy subiculum of that color. Weir distributed specimens of this under the name *F. putearius*, although his type collection of that species is *F. nigrolimitatus*.

Bresadola apparently regarded this species as properly referred to *Fomes spongiosus* (Pers.) Cooke, as there are specimens at the New York Botanical Garden so identified by Bresadola. Romell (Arkiv för Botanik **11**[3]: 18. 1912) indicates that Persoon, Fries, and Secretan each had different conceptions of *F. spongiosus* and each idea has some adherents. It seems best, therefore, for the present, to think of that name as a permanent source of confusion and to rename our plant.

Polyporus transmutans Overholts, sp. nov.

Pileus sessilis, mollis spongiosusque, firmus siccatus, plus minusve ceraceus, non fragilis, albus vel subluteus, subrufus et nigrans contusus, subrufus siccatus; contextus albus, zonatus, 0.5–1 cm. crassus; tubuli 3–5 mm. longi; pori subangulati, 2–4 in uno mm.; sporae oblongae, leves, hyalinae, 5–7 × 2.5–3 μ; cystidia nulla; hyphae contextus nodoso-septatae, 4–7 μ crassae. Specimen typicum in herbario Overholtsii conservatum, no. *22971*. Legit W. A. Campbell et R. W. Davidson in Westline, McKean Co., Pennsylvania, Sept. 10, 1940.

Pileus sessile, soft and spongy when fresh, not bending without breaking, drying firm, brittle, white to very pale buff, drying dark rufescent brown and discoloring dark rufescent on handling, soft tomentose to hispid in part, azonate, 2.5–5 × 3–6 × 1–2 cm.; context white, soft, drying discolored but not hard, zonate in both fresh and dried condition, 0.5–1 cm. thick; pore surface creamy to very pale buff, rufescent or brownish with a tinge of reddish or lavender where handled and in drying, the tubes 3–5 mm. long, their mouths subangular, rather thick-walled, 2 to 2.5 per mm.; spores oblong or short cylindric, smooth, hyaline, 5–7 × 2.5–3 μ; cystidia none; [hyphae rarely branched, with clamp connections, 4–7 μ in diameter]; odor strongly fungoid; taste bitter.

On log of dead *Prunus serotina*. Type collected at Westline, McKean Co., Pa., Sept. 10, 1940, by W. A. Campbell and R. W. Davidson, Overholts Herb. No. *22971*.

This species has some of the characters of *P. fissilis* but is a smaller species with different spores. The cultural characters of this species are almost identical with those of *P. subcartilagineus* but the species is more definitely pileate and the context is much thicker.

PERENNIAL POLYPORES OF NORTH AMERICA. FOMES WITH COLORED SPORES

JOSIAH L. LOWE

(WITH 2 FIGURES)

The perennial pileate polypores of North America have been comprehensively treated by Murrill (**1914**, **1915 a**, **b**, **c**), whose work has been critically reviewed by Lloyd (**1915**). The temperate zone species have been restudied by several modern mycologists and are well known; the tropical species on the other hand need reevaluation with particular reference to the microscopic characters. The present paper deals only with the species referred by Murrill to the genus *Fulvifomes*, an easily ascertained group because the sporophores almost invariably have abundant spores.

Specimens determined by Murrill were studied, and were supplemented by other important collections. The material was sectioned free-hand and mounted in 2 percent KOH. The texture of the context was determined with a needle under low magnification.

The gross morphological characters, such as general shape of the sporophore, shades of color of the context, and the presence or absence of setae, proved so variable that they are considered to be of limited usefulness. Spore size appears to be constant within narrow limits, and setal shape likewise appears to be relatively uniform. In this series of species *Fomes badius*, *F. calcitratus*, *F. extensus*, *F. juniperinus*, *F. melanodermus*, *F. pracrimosus*, *F. sublinteus*, and *F. swieteniae* appear to be distinctive. *F. pectinatus* is very similar to *F. ribis* and may be only "the tropical form" of it, as Lloyd (**1915**, p. 253) believes. *F. linteus* and *F. pseudosenex* are scarcely distinguishable from each other in the first year of growth. In age, setulose forms of *F. linteus* scarcely differ from *F. everhartii* except in the nature of the upper surface, and asetulose forms closely resemble *F. rimosus*. *F. pseudosenex* and *F. dependens* likewise become very similar. On the basis of the type

specimens the latter two species seem sharply distinct from each other; in a series of specimens surely belonging to this complex, however, the specific distinctions intergrade to such an extent that clarification may have to await a comparison of cultural characteristics.

This paper recognizes 15 species. The occurrence of *Fomes melanodermus* var. *tomentosus* in North America is reported for the first time, and the following are reduced to synonymy:

Fomitiporella johnsoniana = *F. dependens*
Pyropolyporus hydrophilus = *F. linteus*
Pyropolyporus jamaicensis = *F. calcitratus*
Pyropolyporus melleicinctus = *F. badius*
Pyropolyporus troyanus = *F. pseudosenex*
Pyropolyporus yucatanensis = *F. linteus*

The loan of specimens by their respective curators is gratefully acknowledged, and is indicated as follows: from the Farlow Cryptogamic Herbarium at Cambridge, Mass., by (F), the Royal Botanic Gardens at Kew, England, by (K), the New York Botanical Garden at New York City, by (NY), the Museum National D'Histoire Naturelle at Paris, France, by (P), and the U. S. Dept. of Agriculture Herbarium at Beltsville, Maryland, by (US). The distribution of 4 species in the United States was adapted from unpublished notes by Dr. L. O. Overholts, namely that of *Fomes everhartii*, *F. juniperinus*, *F. praerimosus*, and *F. rimosus*.

KEY TO THE SPECIES

1a. Context orange-red; on living *Juniperus*.....*Fomes juniperinus*
1b. Context brown; setal hyphae present...*F. melanodermus* var. *tomentosus*
1c. Context brown; setal hyphae lacking
  2a. Sporophore thin, conchate to applanate; spores minute, usually not exceeding 4 μ in longest dimension (compare also *F. dependens*)
    3a. Setae present; sporophore remaining very thin.....*F. extensus*
    3b. Setae lacking; sporophore conchate to applanate
      4a. Context duplex; pileus usually broadly sulcate, becoming large.... *F. ribis*
      4b. Context homogeneous; pileus very narrowly sulcate, small or medium sized.....*F. pectinatus*
  2b. Sporophore becoming applanate to ungulate; spores almost always 4 μ or longer in longest dimension

5a. Spores very dark brown to brownish-black in KOH....*F. sublinteus*
5b. Spores pale yellowish to dark reddish-brown in KOH
6a. Spores averaging more than 5 μ in longest dimension
7a. Pores 2–5 per mm.; tube layers indistinct; often on living hardwoods.....*F. badius*
7b. Pores 7–9 per mm.; tube layers very distinct; on dead wood....*F. calcitratus*
6b. Spores rarely more than 5 μ in longest dimension
8a. Hymenial layer yellow or orange in KOH; spores pale yellow.....*F. praerimosus*
8b. Hymenial layer hyaline; spores usually dark reddish brown
9a. Context hyphae nonseptate or only occasionally septate, solid or thick-walled
10a. Sporophore at first applanate or resupinate, becoming very hard, heavy, and ungulate; surface scarcely becoming rimose
11a. Setae usually present; tramal hyphae 2–4 μ in diam.; spores 3.5–4 × 2.5–4 μ.....*F. dependens*
11b. Setae rarely present; tramal hyphae 4–5 μ in diam.; spores 4–5 × 3–4.5 μ.....*F. pseudosenex*
10b. Sporophore usually convex to ungulate from the first, of medium weight; surface usually soon rimose..*F. rimosus*
9b. Context hyphae frequently to often septate, usually rather thin-walled
12a. Setae usually present; context fibrous, shining, deep reddish-brown
13a. Fruiting body typically ungulate, glabrous; pores 4–6 per mm.....*F. everhartii*
13b. Fruiting body applanate to somewhat ungulate, tomentose, in age matting; pores typically 6–8 per mm.....*F. linteus*
12b. Setae usually lacking; context not fibrous, paler
14a. Surface concentrically zonate.....*F. pseudosenex*
14b. Surface not at all zonate.....*F. swieteniae*

ANNOTATED LIST OF SPECIES

badius Berk. (*Fomes*). Ungulate, becoming rimose; pores 2–5 per mm.; tubes in indistinct layers; context brown; setae usually lacking, or rare, subulate, 25–30 × 5–6 μ; spores pale brown to dark reddish brown, smooth, oval to subglobose, 5.5–7 × 4.5–6 μ. It is very similar to *F. rimosus* but has larger pores and spores. On living hardwood trees or on dead wood in Texas (US), Mexico (NY), the Bahamas (NY), Cuba (NY), and Puerto Rico (NY); also locality uncertain (K-type). *Pyropolyporus melleicinctus* and *P. underwoodii* are synonyms. The paratype of *P. swieteniae* (Brace *1389*) is referred here.

calcitratus (Berk. & Curt.) Cooke (*Fomes*). (FIG. 2, A, B.) Applanate to convex, velvety to glabrous, sulcate; context brown, with a well-developed black line above; tubes cartilaginous, in distinct layers, the pores 7–9 per mm.; setae ventricose, 13–25 × 5–8 μ; spores pale yellowish-brown or in part becoming dark reddish-brown, smooth, subglobose, 5–6 × 4–5 μ. On dead wood in Cuba (K-type; cotypes—F and NY), Jamaica (NY), and Trinidad (K). *Pyropolyporus jamaicensis* is a synonym. The distinctly layered tubes which appear cartilaginous, and the large spores characterize the species.

cedrelae Murr. (*Pyropolyporus*). A synonym of *Fomes rimosus*. (NY-type.)

cinchonensis Murr. (*Pyropolyporus*). Cotype material (US) is sterile, but spores are probably hyaline, as Lloyd (**1915**, p. 242) believes.

demidoffii Lév. (*Polyporus*). An older name for *F. juniperinus*, according to Lloyd (**1915**, p. 279).

densus Lloyd (*Fomes*). A thick heavy form of *Fomes conchatus* (Pers. ex Fries) Karst. with hyaline spores. Overholts' concept of this species is here referred to *F. dependens*.

dependens (Murr.) Sacc. & Trott. (*Fomes*). (FIG. 1, D.) Often at first resupinate, by production of successive tube layers forming an ungulate, pendent pileus, the surface black, sulcate with the annual layers; context and tubes brown, hard and heavy; pores 8 ± 1 per mm.; context hyphae thick-walled, non-septate; tramal hyphae 2–4 μ in diam.; setae ventricose, 11–22 × 5–9 μ; spores pale brown, smooth, broadly oval to subglobose, 3.5–4 × 2.5–4 μ.

On wood in Ontario (U. Mich.), Michigan (NY), Iowa (U. Iowa), D.C. (US), Virginia (US), Louisiana (Overh. Herb.), and Puerto Rico (US-cotype). *Fomitiporella johnsoniana* is a synonym. The concept of *Fomes densus* held by Overholts (Mycologia **23**: 126. 1931), and the specimens distributed by Weir under the name of *Poria pereffusa* belong here. The species is very close to *F. pseudosenex*, and may eventually have to be united with it. See comments under that species.

earlei Murr. (*Pyropolyporus*). A synonym of *F. juniperinus*. (NY-type.)

everhartii (Ell. & Gall.) von Schrenk & Spaulding (*Fomes*). Ungulate, glabrous, becoming rimose; context brown; tubes in indistinct layers, the pores 4–6 per mm.; context hyphae thin-walled, septate; setae 13–35 × 6–11 μ; spores dark reddish-brown, smooth, subglobose to globose, 4–4.5 × 3–3.5 or 4–5 μ in diameter. Usually on living trees; from N. H. and Ontario southward and westward to the Plains, and in Montana.

extensus (Lév.) Cooke (*Fomes*). (FIG. 1, C.) Applanate, remaining very thin; tubes in indistinct layers, the pores 8–11 per mm.; context brown, with a thick black line above; setae ventricose, 13–19 × 7–8 μ; cotype collection sterile, other material with spores pale yellowish-brown, smooth, subglobose, 3.5–4 × 3 μ. On wood in Guadeloupe (P-type; NY-cotype) and Montserrat (NY). The extremely thin pileus and the minute spores characterize the species. It appears to be a rare plant. As Lloyd states (**1915**, p. 248), most of Murrill's determinations are referable to *F. pseudosenex*.

grenadensis Murr. (*Pyropolyporus*). A probable synonym of *F. linteus*, which see. (NY-type.)

hydrophilus Murr. (*Pyropolyporus*). A synonym of *F. linteus*. (NY-type.)

jamaicensis Murr. (*Pyropolyporus*). A synonym of *F. calcitratus*. (NY-type.)

johnsoniana Murr. (*Fomitiporella*). A synonym of *F. dependens*. (NY-type.)

juniperinus (von Schrenk) Sacc. & Syd. (*Fomes*). Ungulate, becoming rimose; pores 1.5–3 per mm.; context orange-red or darker; setae none; spores pale reddish- or olive-brown, somewhat oblong or appearing truncate, 5–7 × 4–5 μ. On living *Juniperus* from Pennsylvania southward and westward to California. *Pyropolyporus earlei* is a synonym, and Lloyd (**1951**, p. 279) states that *Polyporus demidoffii* is an older name for this plant.

langloisii Murr. (*Pyropolyporus*). A synonym of *F. ribis*. (NY-type.)

linteus (Berk. & Curt.) Cooke (*Fomes*). (FIG. 1, B.) Applanate to somewhat ungulate, at first tomentose, the tomentum matting and the surface becoming somewhat rimose; context silky reddish-brown, distinctly fibrous; tubes in indistinct layers, soft,

papery, the pores very variable, usually 6–8 per mm. but as large as 4 per mm.; hyphae thin-walled, frequently septate; setae ventricose, 13–25 × 5–9 μ; spores pale brown to dark reddish-brown, smooth, broadly oval to globose, 4–5 × 3.5–5 μ. On wood in Nicaragua (K-type; F- and NY-cotypes), Yucatan (NY; US), Bahamas (NY), Cuba (NY), Jamaica (NY), and Trinidad (US). *Pyropolyporus hydrophilus* and *P. yucatanensis* are synonyms. *Pyropolyporus grenadensis* appears to be an old, slow-growing example of this species with well-marked tube layers.

melleicinctus Murr. (*Pyropolyporus*). A synonym of *F. badius*. (NY-type.)

melanodermus (Pat.) Sacc. & Syd. var. *tomentosus* Bres. (*Fomes*). Applanate, the surface tomentose, persistently so a long time on upraised zones, eventually glabrous, sulcate; context brown; tube layers indistinct, the pores 8–9 per mm.; context and trama with numerous setal hyphae (FIG. 1, E); setae ventricose, 16–25 × 5–8 μ; spores reddish-brown, smooth, oval, 4.5–5 × 3.5–4 μ. On dead wood in Mexico (NY) and Costa Rica (US). Not reported previously in North America. It is unique in the present series in having setal hyphae.

The variety differs so much from the species (F-type) that it could be considered a distinct plant. The surface of the type of the species is glabrous, with a well-developed crust, and is much less distinctly sulcate; setal hyphae are nearly lacking in the context; and the spores are pale yellowish-brown, 3–4 μ in diameter. A taxonomic change does not appear warranted, however, unless supported by the examination of more specimens.

Field notes on this variety were published by Murrill (N. Y. Bot. Gard. Bull. **8**: 150. 1912) under the name *Pyropolyporus linteus*.

pectinatus (Klotzsch) Cooke (*Fomes*). (FIG. 2, C.) Thin, conchate to applanate, narrowly and concentrically sulcate, up to 4 × 7 × 1 cm.; context brown, homogeneous; pores 10 ± 2 per mm.; setae lacking; spores pale brown, smooth, subglobose to globose, 2.5–3.5 μ in diameter. On dead wood in Mexico (NY), British Honduras (NY), Cuba (NY), and Jamaica (NY); East Indies (K-cotype). *Pyropolyporus subpectinatus* is a synonym.

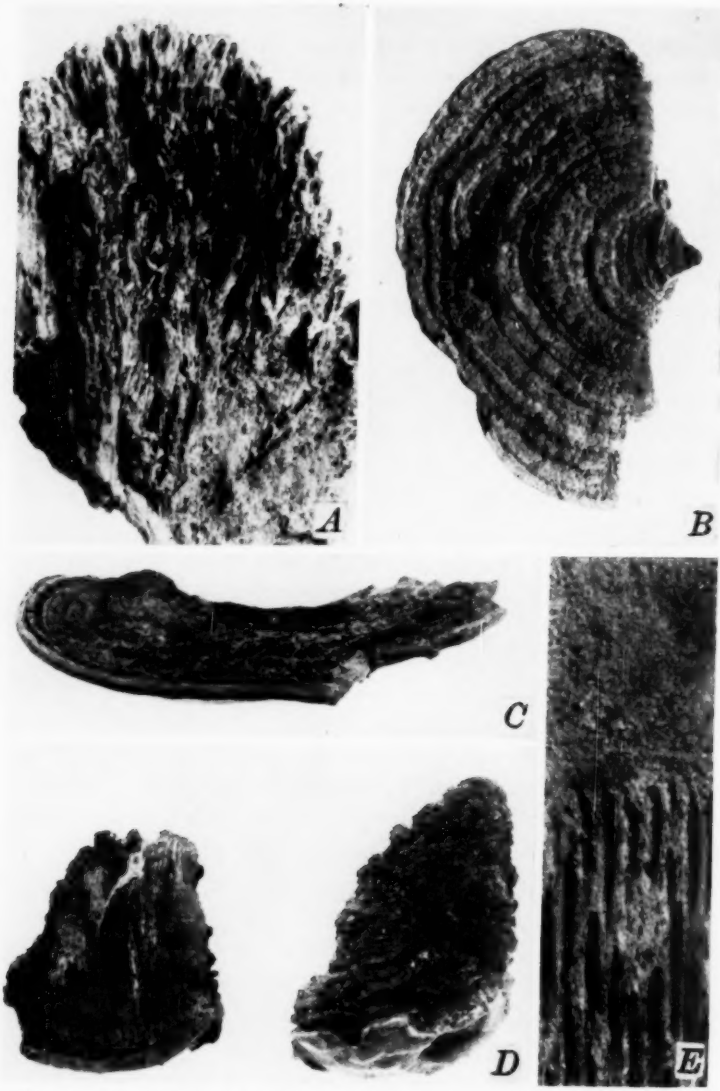

FIG. 1. A. *Fomes sarcitus*, upper surface of type material, × 5. B. *F. lineus*, upper surface of type, × 1. C. *F. extensus*, showing the extremely thin applanate sporophore, × 1. Shafer *876*. D. *F. dependens*, cotype, × 1. E. *F. melanodermus* var. *tomentosus*, longitudinal section of sporophore, × 20. The setal hyphae appear as dark spines in the context and tramal tissue. C. L. Smith *228*.

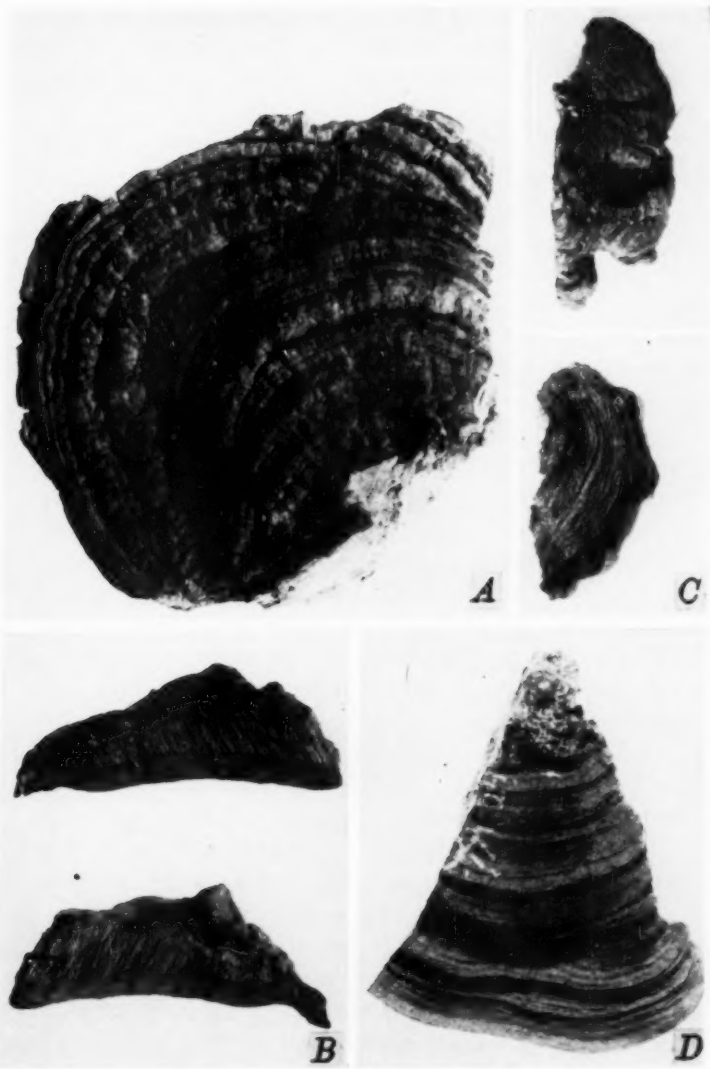

FIG. 2. A. *Fomes calcitratus*, upper surface of type, × 1. B. *F. calcitratus*, vertical section of sporophore showing distinct tube layers, × 1. C. *F. pectinatus*, upper surface of co-type material, × 1. D. *F. pseudosenex*, characteristic upper surface of old incrusted sporophore, × 1.

As Lloyd states (**1915**, p. 253), the sporophore is very similar to that of *F. ribis*, but appears to be sufficiently distinct in the relatively small size, numerous narrow sulcations, and homogeneous context.

praerimosus (Murr.) Sacc. & D. Sacc. (*Fomes*). Ungulate, becoming rimose; context brown; tubes in indistinct layers, the pores 5–6 per mm.; setae ventricose, 21–28 × 6–8 μ; hymenial layer yellow or orange in KOH; spores pale yellow, smooth, oval to subglobose, 4.5–5.5 × 4–5 μ. On living trees in Texas, Arizona, and New Mexico. This species is very similar to *F. everhartii* but differs in the consistently pale spores and colored hymenial layer.

pseudosenex (Murr.) Sacc. & Trott. (*Fomes*). (FIG. 2, D.) At first applanate, thickening and often becoming ungulate, the surface at first velvety, usually soon glabrous and developing a distinct, zonate crust; context at first bright yellowish-brown, darkening and becoming extremely hard and heavy, mottled; tubes indistinctly stratified, the pores 9 ± 2 per mm.; context hyphae at first thin-walled and septate, in age thick-walled and septa inconspicuous; tramal hyphae thick-walled, 4–5 μ in diam.; setae lacking in type and in most specimens, in two collections here referred subulate, 16–32 × 5–8 μ; spores pale brown to more commonly dark reddish-brown, smooth, broadly oval to subglobose, 4–5 × 3–4.5 μ. *Pyropolyporus troyanus* is a synonym. On dead wood in Florida (NY), Mexico (NY), Nicaragua (US-cotype; NY), British Honduras (NY), Panama (U. Tenn.), Cuba (NY), Bahamas (NY), Puerto Rico (NY), Jamaica (NY), and Trinidad (NY).

Young specimens of this species are very troublesome to identify. They merge in their characteristics with asetulose specimens of *F. linteus* and somewhat uncertain differentiation is made on the basis of the not at all fibrous texture of the context. Older specimens often closely resemble sporophores of *F. dependens*, from which the present species is separated by the generally larger, more deeply colored spores, and larger tramal hyphae. When an extensive series of specimens is available for study, it may be found that *F. pseudosenex* and *F. dependens* are synonymous, but the separation at present seems justified.

ribis (Schum. ex Fries) Gill. (*Fomes*). Applanate, usually broadly sulcate, up to 12 × 20 × 1.5 cm.; context brown, duplex,

soft and spongy above, with a separating dark line; tubes in indistinct layers, the pores 5–8 per mm.; setae lacking; spores pale brown, ellipsoid to subglobose, 2.5–4 × 2.5–3 μ. At the base of cultivated *Ribes* or rarely on a few other hosts from southern Canada to Maryland and westward to the prairies, in Louisiana, Montana, and Oregon. *Pyropolyporus langloisii* is a synonym. The sporophore is very similar to that of *F. pectinatus*, but the species seems distinct.

rimosus (Berk.) Cooke (*Fomes*). Applanate to ungulate, usually soon becoming rimose; context brown; tubes in indistinct layers, the pores 5–6 per mm.; context hyphae thick-walled, occasionally septate; setae lacking; spores dark reddish-brown, smooth, broadly oval to subglobose, 4–5 × 3.5–4.5 μ. On living trees of *Robinia*, *Acacia*, and other hosts from New York south and west to the Mississippi and in Iowa, Missouri, Texas, New Mexico, Arizona, California, and in San Domingo and Jamaica. *Pyropolyporus robiniae* and *P. cedrelae* are synonyms.

robiniae Murr. (*Pyropolyporus*). A synonym of *Fomes rimosus*.

sarcitus (Fries) Cooke (*Fomes*). The type collection at Upsala, Sweden, has been kindly examined by Dr. John Eriksson, who writes that no pores and no hymenium are present on the specimen, in agreement with part of the type at the New York Botanical Garden. The species is therefore of quite uncertain relationships, but should be easy to recognize by its peculiar fibrose-lacerate upper surface (FIG. 1, A). The collection reported by Lloyd (**1915**, p. 257) from Trinidad, is *Fomes linteus*.

senex (Nees & Mont.) Cooke (*Fomes*). The type specimen at Paris, from Juan Fernandez Id., is sterile. Lloyd (**1915**, p. 259) states that the spores are colored, but it seems probable that, when found, they will prove to be hyaline.

There are two current concepts of this species, characterized most readily by their setae. The type has ventricose setae with straight tips, 19–25 × 8–10 μ. A specimen labelled "*Fomes senex*, type, Paris" at the New York Botanical Garden has hooked setae, as does another specimen from Africa. These two collections may be referred to *Fomes robinsoniae* (Murr.) Sacc. & Trott.

sublinteus (Murr.) Sacc. & Trott. (*Fomes*). Applanate, very light in weight, with a black smooth crust; pores 6–7 per mm.;

context bright yellowish-brown; setae lacking; spores in KOH very dark brown or brownish-black, smooth, oval with one side usually somewhat flattened, 4.5–5 × 3.5–4.5 μ. On wood in Mexico (NY-type) and Jamaica (NY).

As Lloyd noted (**1915**, p. 285), only one layer of tubes is present and the position of the species in *Fomes* is dubious. It is the only species in the present series with brown-black spores in KOH mounts.

subpectinatus Murr. (*Pyropolyporus*). A synonym of *F. pectinatus*. (NY-type.)

swieteniae (Murr.) Rick (*Fomes*). Applanate, light in weight, the surface pubescent, matting but scarcely forming a crust; context bright yellowish-brown, becoming dull and pale reddish-brown, not fibrous, the tubes in indistinct layers; pores 5–8 per mm.; context hyphae thin-walled and conspicuously septate, 3–7 μ in diameter; setae lacking; spores reddish-brown, smooth, oval to subglobose, 4–5 × 3.5–5 μ. On wood in Florida (NY; US) and Cuba (NY-type). The paratype, Brace *4389* (NY), belongs to another species, *F. badius*.

troyanus Murr. (*Pyropolyporus*). A synonym of *F. pseudosenex*, according to the paratype, Murrill & Harris *1051* (NY), the only specimen seen.

underwoodii Murr. (*Pyropolyporus*). A synonym of *F. badius*. (US-cotype.)

yucatanensis Murr. (*Pyropolyporus*). A synonym of *F. linteus*. (US-cotype.)

STATE UNIVERSITY OF NEW YORK,
COLLEGE OF FORESTRY,
SYRACUSE, NEW YORK

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NOTES ON THE TAXONOMY OF THE ACHLYA FLAGELLATA, ACHLYA IMPERFECTA COMPLEX [1]

T. W. Johnson, Jr.

In **1923**, Coker described two new species of *Achlya*, *A. imperfecta* (based on von Minden's (**1912**) *A. debaryana* var. *intermedia*) and *A. flagellata*. According to Coker, these two species could be differentiated from one another on the following bases: First, the androgynous antheridial branches (arising from the oogonial stalks) of *A. imperfecta* were lacking in *A. flagellata*. Secondly, the former species generally had smaller oospores than did the latter. Third, *A. imperfecta* possessed shorter, less profusely branched antheridial hyphae than did *A. flagellata*. Of this characteristic, however, Coker stated (**1923**, p. 117) that these branches were, in *A. imperfecta*, ". . . often long and complex. . . ." Fourth, the oospheres of *A. imperfecta* only rarely matured to become the characteristic eccentric oospores, whereas the oospheres always matured in *A. flagellata*. With respect to other characteristics, it would seem that these two species are equivalents. Both species possess proliferating oogonia, variable oogonial wall pitting, a preponderance of diclinous over monoclinous antheridial branches, and they are very similar in the nature of the vegetative mycelium and characteristics of the asexual reproductive structures.

Twenty-one isolates of achlyoid fungi recently studied present characteristics intermediate between, or overlapping with, the strictest interpretations of the two aforementioned species. From the morphology of these forms, it is apparent that there is no longer a clear-cut differentiation between *A. imperfecta* and *A. flagellata*, and that they should be considered synonymous. It is the purpose of this paper to establish not only this synonymy, but also to effect the reduction of two other taxa in this complex.

[1] Contribution No. 943 from the Department of Botany, University of Michigan.

TABLE I

Characteristic	*A. flagellata* sensu Coker	Forms							*A. imperfecta* sensu Coker
		433	*8 503*	*436*	*509*	*528*	*5*	*681*	
Maturation of oospheres	Always	Rarely	Always	Rarely	Always	Rarely	Usually	Always	Rarely
Oospore number	2–6	4–8	2–8	2–6	4–8	2–6	2–4	2–6	4–6
Oospore size	28 μ	26–28 μ	20–24 μ	24–26 μ	19–21 μ	26–29 μ	26–29 μ	24–28 μ	19–20 μ
Oogonial size	48–75 μ	44–61 μ	45–73 μ	61–78 μ	42–57 μ	52–61 μ	50–69 μ	42–51 μ	40–45 μ
Wall pitting	Variable	Variable	Variable	Variable	Variable	Variable	Variable	Variable	Variable
Androgynous antheridial branches	Lacking	Present	Present	Lacking	Present	Lacking	Present	Present	Present
Angular oogonia	Present	Present	Lacking	Present	Lacking	Lacking	Lacking	Present	Present
Asymmetrical oogonia	Present	Present	Lacking	Lacking	Lacking	Present	Present	Present	Lacking
Degree of branching of antheridial hyphae	Profuse	Moderate	Profuse	Profuse	Sparse	Moderate	Sparse	Moderate	Sparse

Comparison of the taxonomically significant characteristics of *Achlya flagellata*, *Achlya imperfecta*, and intermediate forms.

The characteristics of eight of the more significant isolates which we have studied are summarized in TABLE I. The features used in this table are for the most part those used by Coker in distinguishing *A. flagellata* from *A. imperfecta*. Other characteristics which seem to us to be useful in separation of these species are also included in these diagnoses. In addition, excerpts from the original descriptions of *A. flagellata* and *A. imperfecta* (Coker, **1923**) are included in this table. The forms are merely designated by the writer's collection numbers.

It is at once obvious from the data in TABLE I that there is considerable overlapping in oospore size of the forms with the sizes given in Coker's descriptions of *A. flagellata* and *A. imperfecta*. This invalidates Coker's separation of these two species on this feature. The same may be said for oospore number and oogonial size. Separation of the two species under discussion on the basis of maturation of oospheres, and the presence or absence of androgynous antheridial branches, is likewise refuted by our forms. Form *528*, for example, has the aborting oospheres characteristic of *A. imperfecta*, but lacks the androgynous antheridial branches which one would associate with aborting oospheres in the identification of the isolate as *A. imperfecta*. Degree of branching of the antheridial hyphae is, as our forms show (and as Coker implied), much too variable to be of any taxonomic value.

The data presented in TABLE I, we feel, need no further clarification or discussion. It is clear from an examination of any given form that whereas it possesses some features limited to the strictest interpretation of *A. flagellata*, for instance, it also exhibits one or more characteristics supposedly found only in *A. imperfecta*. The lacunae between Coker's two species have in our belief been filled by the isolates which we have examined.

In all of the taxa studied wherein abortion of oospheres occurred, we have succeeded in inducing complete maturation of these structures by propagation of the isolates in the oak leaf decoction suggested by Couch (see Ziegler and Linthicum, **1950**), and also in the medium suggested by the latter authors. This is certainly evidence of the inadvisability of using oosphere maturation as a major distinguishing characteristic between the two species in question.

It seems to us that there is only one practical disposition for the forms described in TABLE I. They must be considered as organisms linking *A. flagellata* to *A. imperfecta*. We can see no practical gain in naming and erecting a number of new taxa based on our forms within a complex of two species which even in their original descriptions show considerable interrelationship. The author is convinced, therefore, that the matter of future identification of *flagellata*- or *imperfecta*-like fungi can best be obtained by combining these two species under one name. The epithet *flagellata* apparently antedates *imperfecta* by reason of the technicality of prior pagination (Coker, **1923**, pp. 116, 118). It is proposed, therefore, that the two species under discussion be designated as synonymous, and that inasmuch as *A. flagellata* has priority, it be maintained as the valid binomial.

Nagai (**1931**) described *Achlya flagellata* var. *yezoensis* as a fungus differing from the species only in the ". . . tardiness or suppression of the oogonial formation. . . ." In all other respects, Nagai could find no difference between his variety and Coker's *A. flagellata*. From Nagai's illustrations, we can only conclude that he was merely dealing with a strain of Coker's species. One isolate which we have studied, Form *681*, shows the same singular suppression of sexual reproductive organs as in Nagai's variety, but in all other respects it agrees with our interpretation of *A. flagellata*. The writer is recommending that the variety *yezoensis* also be considered synonymous with *Achlya flagellata*.

Achlya androcomposita Hamid (**1942**) was erected as a new species on the basis of: 1) unpitted oogonial walls; 2) "compound" diclinous, monoclinous, and androgynous antheridial branches; 3) oogonial and oospore size; 4) oospore number (4–11, generally 6–8), and 5) length of zoosporangia. The antheridial branch origin of *A. androcomposita*, as well as its oospore size and number, is quite characteristic of the strictest interpretation of *A. imperfecta*. Ooogonial size of Hamid's species is, however, nearer that of Coker's *A. flagellata*. From Hamid's inadequate illustrations, it seems more than likely that he merely had a form of *A. flagellata*. The paramount difference between *A. androcomposita* and Coker's species is the centric oospores in the former, and eccen-

tric ones in the latter. From descriptions of other water molds by Hamid, we seriously question that he even recognized an eccentric oospore! The unpitted oogonial walls of *A. androcomposita* may very well have been reported on the basis of observations of only those oogonia without wall pitting. Lack of pits is very frequent in oogonia of the forms of *A. flagellata* which we have examined.

The similarities of *A. flagellata*, *A. imperfecta*, *A. flagellata* var. *yezoensis*, and *A. androcomposita* are immediately apparent when one compares the foregoing data with the descriptions and illustrations of these taxa. It is proposed, therefore, that the description of *A. flagellata* be emended to include the latter three taxa, and that the forms which we have described above be considered strains of this species:

Achlya flagellata Coker, Saprolegniaceae 116, *Pl. 37*. 1923.

Achlya debaryana var. *intermedia* Minden, Krypt.-Fl. Mark Brandenburg **5**: 545. 1912.

Achlya imperfecta Coker, Saprolegniaceae 118, *Pls. 38, 39*. 1923.

Achlya flagellata var. *yezoensis* Ito & Nagai, Journ. Fac. Agr. Hokkaido Imp. Univ. **32**: 22, *Pl. 4, fig. 2, Pl. 5, figs. 20–22*. 1931.

Achlya androcomposita Hamid, Proc. Indian Acad. Sci., Sect. B. **15**: 209, *Pl. 2*. 1942.

Mycelium limited or extensive, usually dense only at periphery of colony; two-week-old colony 1–2 cm. in diameter; principal hyphae stout, sparingly branched, 25–115 μ in diameter at base, much-branched at their apices. Gemmae abundant, filiform or irregular, rarely pyriform; terminal or intercalary, single or catenulate; occasionally disarticulating; functioning as zoosporangia. Zoosporangia abundant, fusiform or naviculate, occasionally filiform; straight, curved, or bent, infrequently irregular; 100–1090 μ long by 17–85 μ in diameter, predominantly 300–600 × 25–40 μ; renewed sympodially, occasionally in basipetalous succession, infrequently cymosely. Zoospore discharge achlyoid, rarely aplanoid or dictyoid; spore cluster not persistent at exit pore. Oogonia abundant, lateral, infrequently terminal, rarely intercalary, very rarely in a discharged zoosporangium; spherical or pyriform, infrequently asymmetrical, very rarely angular, doliform, or filiform; 25–135 μ, predominantly 40–70 μ in diameter; immature ones

freely or infrequently proliferating; in some colonies, latent in development. Oogonial wall pitted, unpitted, or pitted only under point of attachment of antheridial cells. Oogonial stalks ½–8, generally equal to twice the diameter of the oogonium in length; stout; usually straight, occasionally bent or curved, very rarely branched. Antheridial branches usually diclinous, occasionally monoclinous, infrequently or rarely androgynous; variable in length and degree of branching; 1 to several on an oogonium, frequently not attached to an oogonium. Antheridial cells tubular, clavate, or irregular; simple or branched; attached by projections, or laterally appressed; fertilization tubes present or lacking, not persistent. Oospheres maturing or not. Oospores eccentric, spherical, occasionally ellipsoidal; usually not filling the oogonium; 1–30, the majority 2–10 in number; 15–45 μ, predominantly 20–28 μ in diameter; mature oospores germinating by a short, unbranched germ tube bearing a small, terminal, fusiform zoosporangium.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN

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WESTERN FUNGI—II [1, 2]

WM. BRIDGE COOKE [3]

(WITH 3 FIGURES)

3. SPECIES FROM EASTERN WASHINGTON AND ADJACENT IDAHO

The following species of fungi have been found during the preparation of "A study of fungi, lichens and bryophytes in six vegetation associations in eastern Washington and adjacent Idaho." It was thought best to present a systematic account of these more unusual species and some novelties in a separate paper in order to leave the "Study" on an ecologic rather than a taxonomic level. Several new species were found in the course of the study, some of which will be described below. The writer is indebted to Miss E. K. Cash for the description and discussion of *Trichoscyphella*, for its Latin diagnosis and for checking the Latin in the other diagnoses; to C. W. Emmons for suggestions concerning *Microsporum;* and to D. P. Rogers for reading the manuscript.

Trichoscyphella tenuipilosa E. K. Cash, sp.n.

Apothecia sessilia, ceraceo-carnosa, subglobosa dein cupulata, albo-pilosa, 1–1.7 mm. in diam., margine involuto et hymenio salmoneo; asci cylindrici, longe stipitati, ad apices rotundati, octospori, 40–55 × 4–5 μ; ascosporae hyalinae, unicellulares, 1–2-seriatae, longe ellipsoideae, 5–6 × 0.7–1 μ; paraphyses hyalinae, filiformes, tenues, simplices, 60–65 × 1–1.5 μ; strato subhymeniali hyalino, ex hyphis hyalinis intertextis composito; excipulum hyalinum prismaticum; pili filamentosi, pertenues, hyalini vel subhyalini, guttulati, 65–85 × 1 μ.

Apothecia sessile, waxy-fleshy, subglobose becoming cupulate, with inrolled margin, triangular to hysteroid when dry, 1–1.7 mm. in diameter, hymenium salmon-buff to ochraceous salmon, apricot orange when dry (Ridgway); exterior white-pilose, tinged with

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[2] Scientific Paper No. 995, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman.

[3] Research Associate in Mycology, Department of Plant Pathology.

salmon where hairs are scarce; asci cylindrical, gradually attenuated to a long stipe, slightly narrowed and rounded at the apex, 8-spored, 40–55 × 4–5 μ; ascospores hyaline, unicellular, mostly obliquely uniseriate, sometimes biseriate, long-ellipsoid, 5–7 × 0.7–1 μ; paraphyses hyaline, slender, somewhat rigid, unbranched, exceeding the asci by 10–15 μ, 60–65 × 1–1.6 μ; subhymenial layer of thin, hyaline, interwoven hyphae, outer excipular layer prismatic, subhyaline; hairs filamentous, slender, subhyaline, not swollen, straight or sometimes twisted or subcircinate, roughened from guttular contents, 65–85 × 1 μ.

On twigs of *Pinus ponderosa*, along California Creek near Valley Ford, Spokane Co., Washington, Oct. 18, 1947, Nov. 5 and 13, 1948 (W. B. and V. G. Cooke nos. *21614* (TYPE), *24718* and *26258*).

Trichoscyphella tenuipilosa may be distinguished from closely related species by its narrower spores and thinner excipular hairs. In the measurements of asci and spores it is close to *Dasyscypha pseudotsugae* Hahn (Mycologia **32**: 138. 1940). The latter species, however, occurs only on cankered areas of *Pseudotsuga*, is accompanied by a conidial stage, and has not been recorded on *Pinus*. G. G. Hahn, who kindly examined a specimen of *T. tenuipilosa*, has reported: "I hesitate to say that the fungus is my *Dasyscypha pseudotsugae* without seeing more material, especially since the latter occurs on cankers, branches and trunks of living trees. Moreover we do not have the imperfect stage which is common with *D. pseudotsugae*." Examination of type material of *D. pseudotsugae*, deposited in the Mycological Collections of the Bureau of Plant Industry, demonstrates that the fungus on *Pinus ponderosa* differs also in the longer and much narrower spores, paraphyses longer than the asci, and in thinner hairs. The same characters of spores and excipular hairs distinguish it also from the unnamed species of *Dasyscypha* described by Bingham and Ehrlich (Mycologia **35**: 295. 1943) on *Pinus monticola*, and from the descriptions of *D. calyciformis* reported on *Pinus ponderosa* and other species of *Pinus* from New Zealand (see Bingham and Ehrlich, Mycologia **35**: 307. 1943).

The above Latin and English diagnoses and the discussion were supplied by Miss Cash. The writer would like to add a few comments on the habitat of the fungus in question. In the grove of

pines in which the fungus was collected are several large colonies of seedling trees around large seed-trees. Apparently because of food shortage in years when snow lies on the ground for long periods, certain rodents girdle the seedlings. The specimens of *Trichoscyphella tenuipilosa* reported above were found on dead bark of crown portions of such injured trees, never on healthy living trees.

Microsporum gypseum (Bodin) Gniart & Grigorakis, Lyon Med. **141**: 377. 1928.

Colonies on buried wool cloth cottony, cream-color; conidiophores 5–30 × 3–5 μ, arising on lateral branches of the mycelium; microconidia pleurogenous on slender lateral branches, ovate, 3–5 × 1.5–2.5 μ; macroconidia ovate, 31–48 × 10–14 μ, 4–7 celled, with warts scattered over the surface; internal and external walls of spore becoming up to 5 μ thick, hyaline; in old cultures; intercalary chlamydospores abundant. C. W. Emmons reports that a subculture of this material agrees best with "Variant A" of this species described in his paper "Pleomorphism and variations in the dermatophytes" (Arch. Derm. Syph. **25**: 987–1001. June 1932).

Throughout the plots used in the "Study," a series of wool cloth strips was planted in the spring of 1949. The cloth was supplied by the Quartermaster Biological Laboratories through the courtesy of E. T. Reese. It was wool charmeen stripped to a standard 6 × 1 inch burial strip. At the end of six weeks in three of the 18 plots several of the strips were covered with sporulating colonies of this species. Directly on the cloth in cream to yellow masses macroconidia were produced in great abundance; in one plot conidiospores of *Thielavia sepedonium* Emmons were also abundant on the strips. The macroconidia were produced at the tips of short lateral hyphal branches; no microconidia were found on the cloth. The macroconidia were not separated from the hypha, nor from the hypha branch (conidiophore), by a septum until after the walls separating the cells had started to thicken. At maturity both the outer wall and the walls of the cells were quite thick, hyaline, and covered with a minute beading. The species was isolated on modified Sabouraud's agar supplied by the Department of Bacteriology and Public Health, State College of Washington, through C. E. Skinner. On this agar the fungus produced abundant microconidia

as well as macroconidia and developed a deep blood-red pigment in the agar. At room temperature it was a week before colonies started to appear after inoculation on agar plates and two weeks before they could be studied adequately. No precaution was taken against infecting the strips with organisms which could cause dermatophytosis prior to burial, and no attempts were made to isolate *Microsporum* from the cloth before burial. A subculture was sent to C. W. Emmons who tested its pathogenicity on guinea pigs with negative results. Since the fungus was picked up in three widely separated areas on only occasional strips of the 24 which were planted in each plot it is thought that this strain of this species may be a naturally occurring soil organism in the region where it was found. The plots in which this organism was found include: woodland dominated by *Pinus ponderosa* with a grass union dominated by *Agropyron spicatum* and *Festuca idahoensis,* on a hillside 4.8 miles south of Stites in the canyon of the South Fork of the Clearwater River, Idaho Co., Idaho, in a relatively undisturbed region given over to mild grazing by cattle; thicket of *Symphoricarpos albus* and *Rosa spauldingii* three miles west of Mohler between Nez Perce and Craigmont in Lewis Co., Idaho; and a thicket dominated by the same shrub species on the north side of the ridge covered by virgin Palouse Prairie vegetation protected on the Campus of The State College of Washington, Pullman, Whitman Co., Washington.

Stromatocrea gen.n.

Sporodochiis cerebriformibus, brunneis; phialidibus palisadibus, simplicis, flavis vel pallide brunneis; conidiis brunneis, sphaericis, parietibus crassis, opacis, subasperulis.

Sporodochia cerebriform, brown; phialids in a palisade, simple, yellow to pale brown; conidia brown, spherical, thick-walled, opaque, subasperulate.

Type species: *Stromatocrea cerebriforme* W. B. Cooke.

The name was chosen at the suggestion of C. G. Shaw because of the stromatic nature of the fructification and its resemblance, according to Miss E. K. Cash and Dr. W. W. Diehl, to a hypocreaceous fructification. To date no perfect stage has been associated with this form genus.

Stromatocrea cerebriforme sp.n., FIGS. 1, 2.

Sporodochiis cerebriformibus, brunneis, 1–2 × 1–3 cm. diam., vel 2 mm. crassis; phialidibus palisadibus, simplicis, flavis vel pallide brunneis, 26.5–37.8 × 6.3–7.6 μ; conidiis brunneis, sphaericis, parietibus crassis, opacis, subasperulis, 8.8–(10.1)–11.5 μ diam.

Hab. in cortice *Aceris glabri* var. *douglasii*.

Sporodochia 1–2 × 1–3 cm. in diameter, up to 2 mm. thick, cerebriform, brown; surface covered with a hymenial palisade of closely packed phialids with developing and mature spores; context white, pseudoparenchymatic; sporodochium originating at a central point, with finger-like projections which are simple or dichotomously branched; hyphae of context hyaline, without clamp connections or other specialized structures; apparently divided into conducting and supporting tissue, the former staining with phloxine and comprising about 25 per cent of the tissue in a section, the latter not staining with phloxine, comprising the remaining tissue

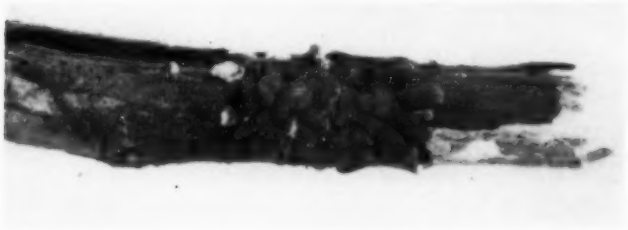

FIG. 1. *Stromatocrea cerebriforme*. Habit, about natural size.

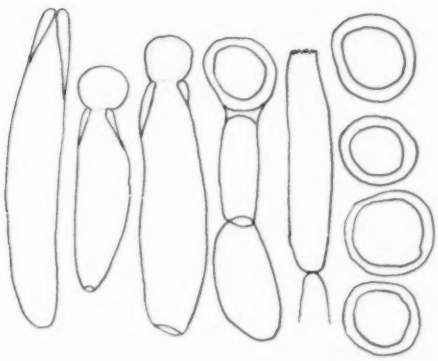

FIG. 2. *Stromatacrea cerebriforme*. Series of phialids with developing conidia; four conidia, × 1000.

in a section; hyphae 4.5–6.3 μ in diameter, densely interwoven but separable; phialids forming a surface palisade, rarely branched once, rarely with a subterminal spore, surface yellowish to pale brown, with a thickened tip which becomes ragged after the spore breaks away, 26.5–37.8 × 6.3–7.6 μ; spores with a thick yellow wall which becomes opaque, completely spherical, nearly smooth but subasperulate under oil powers although appearing slightly sculptured under the high power, 8.8–(10.1)–11.5 μ in diameter.

Habitat: On bark and dead branches of *Acer glabrum* var. *douglasii*, Thatuna Ridge plot, Latah Co., Idaho, W. B. & V. G. Cooke *23152*, March 28, 1948; and Springdale plot, Stevens Co., Washington, W. B. & V. G. Cooke *20740* (TYPE), Sept. 27, 1947, and *22512*, Nov. 8, 1947.

Ascochyta lomatii sp.n.

Maculis epiphyllis, indeterminatis, brunneis; pycnidiis pseudoparenchymatis, ostiolatis, 250 μ diam.; pycnidiosporophoris 20–25 × 8–10 μ; pycnidiosporulis hyalinis, cylindraceis, medio 1-septatis, vix constrictis, 19.8–23.4 × 6.5–7.5 μ.

Hab. in foliis *Lomatii grayi*.

Spots indefinite, brown, covering finely dissected portions of leaves; pycnidia embedded in spots, concolorous with them, difficult to see, pseudoparenchymatous, ostiolate, not beaked, about 250 μ in diameter; inside of pycnidium lined with pycnidiosporophores 20–25 × 8–10 μ; spores produced singly, not appearing in chains; pycnidiospores hyaline, 0–1-septate, constricted at the septum, 1–2-celled at maturity, 19.8–23.4 × 6.5–7.5 μ.

Habitat: On leaves of *Lomatium grayi:* W. B. & V. G. Cooke *23457* (TYPE), May 28, 1948, in *Symphoricarpos albus-Rosa spauldingii* association, virgin Palouse Prairie plot, The State College of Washington Campus, Pullman, Whitman Co., Washington. Also from the same location but in the *Festuca idahoensis-Agropyron spicatum* association: W. B. & V. G. Cooke *23478*, May 28, 1948.

This species has larger pycnidia and spores than *A. heraclei* Grove which appears to be the only species of *Ascochyta* in the literature on an umbelliferous host.

Associated in the spots on these leaves are conidiophores and conidia of a fungus identified as *Ramularia heraclei* (Oud.) Sacc. In this case, the conidiophores of the fungus tend to move out onto the gland cells of the leaf margins from which they produce their conidia.

Helminthosporium pseudotsugae sp.n., FIG. 3.

Effusum, nigrum, dense floccosum vel crustosum; hyphis longissimis intricatis, simplicibus vel ramosis, laxe septatis, 8–12 μ crassis, olivaceoatris; conidiis fusoideo-cylindraceis, acro-pleurogenis, 8–14-septatis, ad septa non constrictis, rectis vel leniter curvulis, 65–105 × 14–15 μ, concoloribus.

Hab. in resina et ad corticem *Pseudotsugae taxifoliae* var. *glaucae*.

Forming a black crust over cracks in the bark and resin exudations on trunks of young trees; hyphae black, loosely to densely matted, 8–12 μ in diameter, with occasional septa, with thick walls; conidiospores produced on very short processes scattered on aerial hyphae with usually one at each cell, spores at maturity opaque, black or greenish black, averaging 14–15 μ in diameter but reaching a length of 65–105 μ, with 9–15 cells, with thick, smooth walls.

Habitat: On bark and resin exudations of *Pseudotsuga taxifolia* var. *glauca* at the following locations: Washington: Stevens Co.: Springdale plot, W. B. & V. G. Cooke *19550*, April 26, 1947; Spokane Co.: 8.2 miles north of Spangle along U. S. Highway 195, W. B. & V. G. Cooke *19570*, April 26, 1947; Idaho: Idaho Co.: Rackliff Creek Ridge Trail one-half mile above Rackliff Creek Public Camp, Nez Perce National Forest, W. B. & V. G. Cooke *18784*, Nov. 13, 1946; *20687*, Sept. 20, 1947; *23000*, March 13, 1948; *26041*, Oct. 22, 1949; Nez Perce Co.: 4.8 miles east of Lenore along the Clearwater River, W. B. & V. G. Cooke, *18947*, Nov. 30, 1946; *22905*, March 13, 1948; *25161* (TYPE), May 14, 1949; Latah Co.: Dry Creek plot, W. B. & V. G. Cooke *19583*,

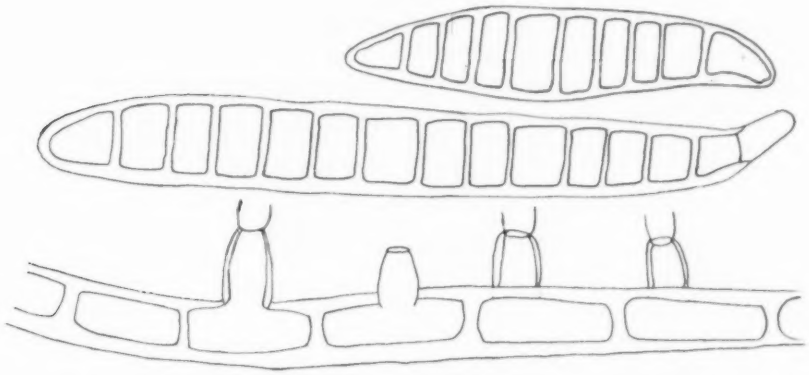

FIG. 3. *Helminthosporium pseudotsugae*. Two mature spores and portion of a conidium-bearing hypha with four spore-bearing cells, on three of which spores are being produced.

April 27, 1947; Thatuna Ridge plot, W. B. & V. G. Cooke *23429*, May 16, 1948.

This species resembles *H. resinae* Bres. described from *Larix europea* and *Abies pectinata* from Italy. While the Italian species was reported to be the imperfect stage of a capnodiaceous fungus, *Limacinia resinae* Bres. & Sacc., our species has not been found with a perfect stage in nature on Douglas Fir although it was sought for in all seasons during which the plots were visited.

Coccodinium laricis W. B. Cooke & C. G. Shaw, sp.n.

Subiculo 1–4 mm. cr., crustaceo, nigro vel brunneo-nigricanti, late effuso, ex hyphis ramosis, 5–8 μ cf., brunneo-nigricanti formato; peritheciis sparsis, sessilibus, globosis, 225–300 μ diam., nigris, opacis; ascis clavato-ovoideis, apice rotundatis, octosporis, 108 × 18 μ; sporis biseriatis, oblongis, brunneis vel atro-brunneis, muriformibus, 10–12-cellularibus, 21–6 × 7.2 μ.

Hab. in cortice *Laricis occidentalis*, Viola, Thatuna Hills, Latah Co., Idaho.

Subiculum crustaceous, 1–4 mm. thick, black to brownish-black, broadly effused, formed from branched hyphae 5–8 μ in diameter, dark brown; perithecia scattered, sessile, globose, 225–300 μ in diameter, black, opaque, ostiole not prominent, provided with hair-like chains of cells, brown, 20–50 × 5–8 μ; asci clavate-ovoid, apex rounded, produced in a basal cluster, thick-walled, 8-spored, 108 × 18 μ; paraphyses not seen but upper part of perithecium with fine hyaline hyphae growing downward toward the asci; spores mostly biseriate, rarely uniseriate, oblong, brown, finally dark brown, muriform, with 5 transverse septa and 1 longitudinal septum, 10–12-celled, averaging 21.6 × 7.2 μ.

Habitat: On bark around old branch base on trunk of *Larix occidentalis*.

TYPE: 2 mi. east of Viola, Thatuna Hills, Latah Co., Idaho, May 21, 1948, C. G. Shaw (WSC-PP *16074*).

Coccodinium was erected by Massalongo (Esam. comp. p. 55. 1860) for a capnodiaceous fungus growing on *Alnus* and *Tilia* in Germany and Austria. It was based on *C. bartschii* which had smooth perithecia with muriform spores which had up to 8 cells and measured 22–36 × 9–12 μ. Theissen & Sydow (Ann. Mycol. **15**: 389–491. 1917) considered this genus synonymous with *Naetrocymbe* Körber, a name used first in 1858 for a specimen distributed in Lich. sel. Germ. n. 58. The name was not formally

published with a description until 1865 when Körber published it in his Parerga lich. p. 441. *Naetrocymbe fuliginea* Körber is therefore considered synonymous with *Coccodinium bartschii* Massalongo by Theissen & Sydow since both genera were monotypic. So far as we have been able to find no other species have been placed in this genus. While this genus is described as not possessing bristles, the hairs we have described on the perithecium are not considered bristles by us since they are not stiff and pointed but flexuous and hypha-like. *Phacosaccardinula* P. Henn. is similar to this but the spores at first are hyaline, then rosy and finally pale brown at maturity. In our material the spores are brown from the inception of wall formation. Saccardo and Bresadola placed a species from southern Europe similar in habit and habitat to this in *Limacinia* Neger. Their species, *L. resinae,* with the imperfect state *Helminthosporium resinae,* differs from ours in having light brown ascospores which are only transversely septate, not muriform. *Phragmocapnias* Theiss. & Syd. is similar to *Limacinia* Neger but the spores are brown instead of hyaline. On this basis, Theissen and Sydow suggest that it is probably a better genus in which to include *L. resinae.* The imperfect state of *L. resinae* was found on pines and firs, while the perfect state was found on larch. In our fungus the black stromatic tissue produces spores assignable to *Helminthosporium pseudotsugae* W. B. Cooke which are very much like those described for *H. resinae* both on *Pseudotsuga* and *Larix.* The perfect state is known only from the collection on *Larix occidentalis.*

Phlebiella candidissima (Schw.) comb.n.

Polyporus candidissimus Schw. Amer. Phil. Soc. Trans. II. **4**: 159

Poria candidissima (Schw.) M. C. Cooke, Grev. **14**: 110. 1886.

Trechispora candidissima (Schw.) Bond. & Sing. Ann. Myc. **39**: 43–65. 1941.

Cristella candidissima (Schw.) Donk in mss. ex W. B. Cooke, Mycologia **35**: 288. 1943.

Rogers (Mycologia **36**: 70–103. 1944) has shown that the genus *Cristella* is not available for use and that *Phlebiella* is the only name which can be used for the group of fungi to which this species belongs. This opinion is accepted by Singer (Mycologia **36**: 65–69. 1944) in defining a number of genera of pore fungi proposed by Bondarzew and Singer (Ann. Mycol. **39**: 43–65. 1941). The combination is suggested in several places but is here published for the first time. Collected twice on rotten conifer wood in a Grand Fir woodland in the Dry Creek plot, Latah Co., Idaho.

Haplographium bicolor Grove

A culture assigned to this species by K. B. Raper was obtained from soil samples collected in an *Abies grandis-Pseudotsuga taxofolia* var. *glauca* woodland on the mountainside a half-mile up Rackliff Ridge Trail from Rackliff Creek Public Camp, Nez Perce National Forest, Idaho Co., Idaho. The conidiophores and spores are golden brown in the colony, yellow-brown to brown in mounts. At the tip of the conidiophore the penicils and phialids are arranged as in *Penicillium*. In our material these occur in groups of three to five. The spores are produced in chains, are roughened by fine apiculations and measure 2.5–3.2 μ in diameter. The hyphae and conidiophores measure 3.5–4 μ in diameter.

Coniosporium shiraianum (Syd.) Bubak in Österr. Bot. Zeitschr. **55**: 244. 1905.

Melanconium shiraianum Syd. Hedw. **38**: (143). 1899.

Coniosporium hysterinum Bubak in Österr. Bot. Zeitschr. **54**: 186. 1904.

Conidiophores short hyphal branches, more or less decumbent, hyaline to yellowish, produced from hyaline hyphae, bearing black conidiospores singly. Spores circular in face view, ovate in side view, surrounded by a Saturn-like ring. Apparently at maturity, or on teasing, the spores open at this ring and the protoplasmic contents escape. Spores 10.5–14 × 7–10 μ. The culture from which this description was drawn was obtained from a soil sample collected in a woodland dominated by *Pinus ponderosa* with *Sym-*

phoricarpos albus and *Rosa spauldingii* in the shrub union. This woodland lies about 10 miles west of Pullman along a tributary of Union Flat Creek in Whitman Co., Washington. *Coniosporium* Lk. ex Fr. 1829, being the genus Fries used in the Systema, is the genus which must be used for species assignable to *Papularia* Fr. 1825 (Syst. Orb. Veg.) and *Coniosporium* Lk. ex Fr. 1825 (l.c.).

Helminthosporium velutinum Lk. ex Sacc.

An isolate from soil collected near the western end of Thatuna Ridge on its north side in a *Pseudotsuga taxifolia* var. *glauca* woodland in Latah Co., Idaho, proved to belong to this genus. In an old culture were found simple, dark, 3–5-septate phragmosporous spores. The spores were dark colored and smooth at maturity. They measured 20–25 × 8–10 μ, and were produced acrogenously and acropleurogenously. In a young transfer some of the spores were as above; others, however, were forked, had swellings in the middle or at one end. In these peculiarly shaped spores there was a tendency for the septation to become dictyosporous with 3–5-septa. These spores measured 25–40 × 7.5–12.5 μ. This species, ubiquitous on all sorts of litter and debris, comes closest to those listed by Lindau as a repository for this fungus.

Stemphylium consortiale (Thüm.) Groves & Skolko

A culture assigned to this species was made from soil samples collected in the virgin Palouse Prairie strip on the Campus of The State College of Washington. The spores are irregular in shape and in septation, measuring 12–32 × 12–15 μ. The spores are roughened at maturity, opaque and nearly black. There is always one septum across the center of the spore. There may be up to three additional septa parallel to this one. Usually at least one of the cells thus formed is divided by a longitudinal septum. There may be two or three such septa in some spores. In some spores the septa may be diagonal. The spores are borne singly on most cells of the conidiophore which is a hypha projecting from the dense mat of dark hyphae which form the colony on the agar. The posi-

tion of the spores on the conidiophore gives this structure a geniculate appearance.

Pestalotia virgatula Klebahn

Material assigned to this genus was isolated from soil samples collected in the virgin prairie strip on the Pullman campus. On the surface of the slant in transfers the fungus developed a white appressed cottony mycelium from which were produced black pustulate masses of conidia. The conidia were blackish with terminal hyaline cells, 4-celled, with 1, 2 or 3 cilia produced at the tip of one terminal cell or on an elongation of the terminal cell. The spores measured 17.5–20 × 5.5–6.5 μ with setae up to 17.5 μ long.

No species of this genus is reported to our knowledge in the literature from soil samples, although elementary classes in isolation technique have picked up such material several times at Pullman in recent years. Assignment to this species is tentative and based on comparison with material so identified from cloth buried in Panama.

4. A SPECIES OF DOTHIDELLA ON FAGACEAE

A number of collections of *Dothidella castanicola* (Ell. & Ev.) Bonar came up for filing in the Plant Pathology Herbarium at The State College of Washington. In the folder we also found material labeled *Dothidella castanopsidis* and *Plowrightia* n.sp., so the literature available to us was checked to discover the correct name for this organism.

Of the three specific names available for this species, assuming that *Dothidella* on western species of *Castanopsis* is different from other species of *Dothidella* on species of Fagaceae, the question arose as to which was applicable to our organism. *Phyllosticta castanicola* Ell. & Ev. and *Dothidella castanicola* (Ell. & Ev.) Bonar were typified by a specimen from Mount Shasta (Sisson), California; *Dothidella castanopsidis* Dearness was based on Oregon material; *Sphaerella weiriana* Saccardo was based on material reported to be from Spokane, Washington, all on species of *Castanopsis*. Of these, *Phyllosticta castanicola* is the oldest; *Sphaerella weiriana* is second oldest.

At the University of California herbarium Lee Bonar reports that two specimens are filed under this complex collected from *Quercus* species, identified by O. A. Plunkett. At the Mycological Collections, Beltsville, one specimen on *Quercus sadleriana* from Grant's Pass, Oregon, is filed under *Dothidella janus* (Berk. & Curt.) Höhnel. This determination was made by W. W. Diehl. One could infer from this that here were two species, one, *D. castanicola*, confined to the West Coast on species of *Castanopsis* and occasionally getting over onto *Quercus;* the other, *D. janus*, usually confined to southern evergreen *Quercus* species and rarely cropping up on western species of this genus. Or one could infer that mycologists working in one region were more familiar with a series of names applied to species in that region than with those applied in another. Since Ellis had good material of both species it is evident that either he did not realize the close relationship between the host genera *Castanopsis* and *Quercus*, or he did not connect these fungus species other than in his filing cabinet.

Through W. L. White, a specimen of *D. janus* was obtained from the Curtis Collection of the Farlow Herbarium. This was collected by Wright from *Quercus* in New Mexico. Aside from the fact that the perithecia are old and sterile this material is typical of symptoms displayed by other material. No material of the type of this species appears to be available in the United States. The type collection was obtained from *Quercus* in Texas and was reported by von Höhnel as sterile. The species was originally reported as having 4-celled colored spores. On this basis Saccardo transferred it to *Leptosphaeria*. Berlese thought it belonged in *Metasphaeria*, although he found it to be sterile. The 4-celled yellow spores could easily have been fragments of the peculiar hairs found on the leaves. Von Höhnel found the Curtis material sterile and apparently based his idea of the species on Ellis's North American Fungi No. *1299* collected by Dr. Martin in 1883 from *Quercus* in Florida. Material of this specimen, and other material of this species at the New York Botanical Garden, was lent by D. P. Rogers. It was found to be fertile with good asci in all stages of development. Two other specimens on oak leaves from Florida were also fertile and both possessed the typical *Phyllosticta* stage.

Both from outer appearance, and from microscopic characteristics, there seems to be not enough difference between this species and the western species to warrant retaining two separate species.

Since this species occurs mostly on species of *Quercus* in the southeastern and southwestern United States, and on evergreen species of *Quercus* and *Castanopsis* on the west coast, as well as on *Quercus sadleriana* (a deciduous shrub endemic to the "Klamath Province" in northwestern California and southwestern Oregon), it appears likely that there is a center of distribution southward in Mexico from which oaks and chinquapins could be infected on both sides of the cordilleran axis.

In picking up Berkeley & Curtis's name for this species the writer is resisting the temptation to make a new combination based on *Sphaerella weiriana*, the oldest name applied to the perfect stage based on western material. While Ellis & Everhart did not observe nor describe mature perithecia, asci and ascospores in *Phyllosticta castanicola*, they are present in their type material. The only mention of such bodies in the original description is as sclerotoid bodies. Thus it was felt that their name was not applicable to the perfect stage, especially when the perfect stage was well described in the literature.

The synonymy of this species becomes:

Dothidella janus (Berk. & Curt.) Höhnel

Sphaeria janus Berk. & Curt. Grev. **4**: 154. 1876.

Leptosphaeria janus (Berk. & Curt.) Sacc. Syll. Fung. **2**: 85. 1883.

Metasphaeria janus (Berk. & Curt.) Berlese, Icon. **1**: 88. 1894.

Phyllosticta castanicola Ell. & Ev. Proc. Acad. Phil. **1895**: 431. 1895.

Dothidella janus (Berk. & Curt.) Höhnel, Sitzungsb. Akad. Wiss. Wien. Math.-Naturw. Kl. **124** (Abt. 1): 67–68. 1915.

Sphaerella weiriana Sacc. Nuovo Giorn. bot. ital. **27**: 76. 1920.

Dothidella castanopsidis Dearness, Mycologia **16**: 155. 1924.

Dothidella castanicola (Ell. & Ev.) Bonar, Mycologia **20**: 294. 1928.

Specimens from California, Oregon, New Mexico and Florida have been lent by D. P. Rogers, New York Botanical Garden, and by W. L. White, Farlow Herbarium. Specimens have been examined from the herbarium of the Dept. of Plant Pathology, The State College of Washington, and from the writer's collection. P. L. Lentz supplied notes on the material on *Quercus sadleriana* filed in the Mycological Collections, Bureau of Plant Industry. Through P. L. Lentz, W. W. Diehl supplied his observations on this material. Lee Bonar has supplied notes and a list of material filed in the Mycological Herbarium at the University of California, Berkeley, and has kindly read this portion of the manuscript.

Based on material and notes mentioned above the host and geographic range of this species becomes: On *Castanopsis chrysophylla:* California: Siskiyou, Humbolt, Mendocino, Fresno and Tuolumne Counties. Oregon: Douglas, Jackson, Klamath, Benton, Clackamas, Marion, Hood River, Jefferson, Linn and Lane Counties. Washington: Spokane County. On *Castanopsis sempervirens:* California: Siskiyou, Lassen, El Dorado, Fresno, Tuolumne and Los Angeles Counties; Yosemite, Sequoia and Kings Canyon National Parks. Nevada: Douglas, Ormsby Counties. On *Quercus* spp.: Texas; New Mexico. On *Quercus dumosa* Nutt.: California: Los Angeles County. On *Quercus geminata:* Florida: Alachua County. On *Quercus sadleriana:* Oregon: (Grant's Pass). On *Quercus virens:* Florida: (Green Cove Springs). On *Quercus virginiana:* Florida: (Keystone Heights).

5. FUNGI OF A LAVA TUBE

At Lava Caves State Park near Bend, Deschutes County, Oregon, one of the lava tubes has been opened to the public. To make the walk through it safer, a hand railing has been installed. The uprights of this railing are of wood, probably of *Pinus ponderosa* branches since that is the dominant, if not the only, tree species in the area. In parts of the tube the railing itself is wire. In other parts it is pine wood. The tube is about a mile long and in some places reminds one of man-made tunnels through which subways run. There is little seepage from the surface, which lies a hundred

feet above the floor of the tube at the entrance. The floor of the tube slopes gently downward and westward. The only lighting in the tube is supplied by flashlights or by gasoline lanterns which are rented to visitors. The caves are well described in a popular article in Natural History, Vol. **23** (1923).

Throughout the length of the tube fungi were observed on the wooden posts and railings. At the sandy soil surface and along the sides of the posts there was an abundant fruiting of *Naematoloma*. There were several species of resupinate Thelephoraceae on the posts. At one point a Discomycete was found and a large number of the *Naematoloma* fruit bodies were infected with a beautiful golden fungus.

Isaria citrina Pers. Forming elongated branched ropes of hyphae on stipes and caps of *Naematoloma* fruiting on pine posts and at the soil line near the posts. The elongated ropes at maturity are golden to lemon color; when young they are white. The ropes are composed of hyaline hyphae which branch to form conidiophores or which terminate in conidiophores toward the tip of the rope. The conidiophores are elongate, hyaline, with slender tips at which are produced minute, hyaline, ovate spores in short chains which readily break up on mounting. W. B. & V. G. Cooke *25713*.

Naematoloma sublateritium (Fries) Karst. Fruiting on pine posts supporting the guard rail. Fruit bodies appear at all points from the ground to about midway up the three-foot posts. The stipes appear to be somewhat longer than those of specimens observed at other times in the field under more normal light conditions. There was no apparent difference in pigmentation of the stipe, gills or cap between those fruiting in the tube and those fruiting in daylight. What appear to be sterile buds occurred over the length of these posts. W. B. & V. G. Cooke *25714*.

Corticium fuscostratum Burt. A specimen, W. B. & V. G. Cooke *24707*, found on the underside of one of the guard rails was thus identified by H. S. Jackson.

Peniophora mollis (Bres. sic!) Bourd. & Galz. W. B. & V. G. Cooke *25708* was found fruiting on the under side of guard rails. Identified by H. S. Jackson.

An immature Discomycete was found in sand near the posts.

It appears like a *Sepultaria* but no asci have developed in the material collected. It would be interesting to know whether or not the lack of asci was the result of response to a negative light stimulus.

A white mold was found on some of the posts on what appear to be undeveloped initials of the *Naematoloma*. Casual observation of dried specimens gives a somewhat unsatisfactory idea of the species but it appears to be a species of *Sporotrichum*. The spores measure $3 \times 1.5\,\mu$. W. B. & V. G. Cooke *25706*.

NOTES AND BRIEF ARTICLES

The status of the generic name Oxydontia L. W. Miller ("Hydnaceae")

In a recent number of *Mycologia* (**43**: 459–462. 1951) M. A. Ragab discussed the status of the generic names *Acia* P. Karst., *Mycoacia* Donk and *Oxydontia* L. W. Miller. As to the nomenclatural side of the note I agree with him. On the other hand I would rather not subscribe to his taxonomic conclusions. To me *Mycoacia* is a genus not to be incorporated in *Odontia* S. F. Gray nor "*Odontia* Fr. sensu Bourdot & Galzin," but related to *Phlebia* Fr. and to certain species of *Corticium* (sensu Bourdot & Galzin), some of which possess leptocystidia. However, this is a subjective matter that need not be discussed here. I do agree as to the conclusion that the type species of *Mycoacia* and *Oxydontia* should be placed in different genera; the relations of the latter genus and of *Donkia* Pilát, *Gloiodon* P. Karst., and *Climacodon* P. Karst. (and a few other hydnaceous fungi) being, I suspect, with quite a different group of Hymenomycetes than those of *Mycoacia*, viz., to a set of genera included in "Polyporaceae," and of which *Spongipellis* Pat. is an outstanding member.

The correct name for the genus including *Hydnum setosum* Pers. is neither *Mycoacia* nor *Oxydontia*. The type of *Mycoacia*, by original designation, is *Hydnum atrofuscum* Fr. This species cannot be replaced by *Hydnum setosum* as was done by Miller & Boyle (Univ. Iowa Stud. Nat. Hist. **18** (*2*): 38. 1943) for some obscure reason. Further, *Oxydontia* is preoccupied by a much earlier, generally overlooked typonym, viz., *Sarcodontia* S. Schulz. The latter name, then, would appear to be the correct one for a not too inclusive genus embracing *Hydnum setosum* but not *Hydnum fuscoatrum*. The reestablishment of *Sarcodontia* as the name of a valid genus necessitates the following combination to be made: **Sarcodontia setosa** (Pers.) Donk, comb. nov. (basinym: *Hydnum setosum* Pers., Mycol. europ. **2**: 213. 1825). The other species

transferred by Miller and Miller & Boyle either to *Mycoacia* or to *Oxydontia* I do not want to include in *Sarcodontia* at the moment, for various reasons.

The following note on this generic name is extracted from a manuscript that will be sent to the printer's during the course of the first half of the next year.

Sarcodontia S. Schulz. apud S. Schulz., Kanitz & Knapp in Verh. zool.-bot. Ges. Wien **16**: Abhandl.: 41. 1866. All that was published about this genus runs:

"SARCODONTIA Schulzer, *Kneiffia* Fr. form b. Stroma d i c k, f l e i s c h i g formlos, auf der ganzen Oberfläche mit ungleichförmigen Zacken besetzt. Gehört weder habituell noch der Substanz nach zu den Telephorei, sondern hieher [Hydnei?].

"295. *S. Mali.* Vinkovce an kranken (veredelten) Aepfeln."

Later on, the author (S. Schulzer von Müggenburg in Verh. zool.-bot. Ges. Wien **24**: Abhandl.: 293. 1874) described the only species on which he founded it more fully under the name of "*Kneiffia setigera* Fr. *β. pomicola* Schulzer, olim *Sarcodontia Mali* mihi." The one and a half pages of closely printed additional information on the type species of the generic name leaves no doubt, I believe, that it represents *Hydnum setosum* Pers. This makes *Oxydontia* L. W. Miller a later typonym.—M. A. DONK.

MICROCOPIES OF MYCOLOGIA

Since 1940 the *Review of Applied Mycology* has performed a great service to workers in mycology and related fields by publishing, in the *Index of Fungi* (formerly *Supplement to the R.A.M.*), citations of all new genera, species, and combinations which have appeared during that period. The Commonwealth Mycological Institute now proposes to issue in connection with the *Index* microcards of descriptions and figures of all new genera and species where permission to reproduce has been given. Those published in *Mycologia* will be included.

As a contribution to the problem of storage in large libraries, University Microfilms, of Ann Arbor, Michigan, is offering microfilm copies of a long list of periodicals to subscribers for the respec-

tive periodicals only. Subscribers to *Mycologia* may wish to avail themselves of this service.—DONALD P. ROGERS.

Kansas State College, Argonne National Laboratory and the Isotopes Division of the United States Atomic Energy Commission will sponsor a two-and-a-half-day conference on "The Use of Isotopes in Plant and Animal Research," June 12–14, 1952, at Kansas State College, Manhattan, Kansas. A detailed program will be available in March. Persons wishing to receive it, together with information on housing, should write Director R. I. Throckmorton, Kansas Agricultural Experiment Station, Manhattan, Kansas.

ECOLOGICAL LIFE HISTORY REPRINTS

Reprints of "Ecological life history outlines for fungi" by William Bridge Cooke, Ecology **32**: 736–748. 1951, may be ordered through the Secretary of the Ecological Society, Dr. Murray F. Buell, Rutgers University, New Brunswick, N. J. Orders should be placed before April 15th. The price will be in the neighborhood of $3 to $4 a hundred.

Dr. Gertrude S. Burlingham, known for her extensive studies on the agarics, died at Winter Park, Florida, on January 11th.

REVIEWS

MYCOLOGICAL FLORA OF JAPAN. VOL. II. BASIDIOMYCETES. NO. 3. UREDINALES-PUCCINIACEAE, UREDINALES IMPERFECTI, by Seiya Ito. 435 pp., 453 figs. Yokendo, Tokyo. 1950.

This volume, written in Japanese, is a systematic account of the Pucciniaceae of Japan. It is similar to Arthur's "Manual" and should prove as useful in Japan as the latter has in North America. Despite the Japanese text, Ito's contribution will be valuable to anyone interested in the rusts of Japan since it is the first complete published record of the Pucciniaceae. Moreover, the synonymy is complete and the line drawings, which accompany the descriptions of most species, are excellent. There is a key to the genera but keys are not provided to the species. The descriptions of species within genera are arranged according to their hosts. A rust index and a host index complete the volume. Considering Ito's "Pucciniaceae" and Hiratsuka's "Melampsoracearum Nipponicum" (Mem. Tottori Agric. College **7**: 9–273. 1944), the records of the rusts of Japan are now as complete and readily accessible as for any area of the world.—GEORGE B. CUMMINS.

THE CHEMISTRY AND PHYSIOLOGY OF PROTOZOA, VOL. 1, edited by Andre Lwoff. 434 pages. Academic Press, New York, 1951. Price $8.80.

This is a collection of seven short monographs by eight authors on the chemistry and physiology of protozoa. The authors have contributed substantially to our knowledge of the subject reviewed. The editor has written an "Introduction to Biochemistry of Protozoa" indicating the scope of the volume. The chapter on Phytoflagellates by S. H. Hutner and Luigi Provasoli (102 pp.) is a compressive review of the subject made in the spirit of the quotation from James Thurber given at the beginning of the chapter. The authors are not parochial in their speculations, which range

from the origin of the Phytoflagellates and the mechanism of C_4-dicarboxylic acid formation by *Rhizopus* to the absence of astaxanthin in the mammalian retinas. The principal criticism of this chapter is the tendency of the authors to forget that their highly speculative explanation of a phenomenon is speculation and not fact. The number of unsolved problems in the chemistry and physiology of the Phytoflagellates discussed here indicates how little is known about these organisms. Marguerite Lwoff writes two chapters, The Nutrition of Parasitic Flagellates (Trypanosomidae, Trichomonadinae, 48 pp.) and Nutrition of Parasitic Amebae (15 pp.). Theodor von Brand, in his discussion of the Metabolism of Trypanosomidae and Bodonidae (57 pp.), devotes much of the chapter to pathological physiology and its relation to chemotherapy. The fascinating story of the "Biochemistry of Plasmodium and the Influence of Antimalarials" is told by Ralph W. McKee in 70 pages. When the difficulty of working with an obligate intracellular blood parasite is considered, the knowledge about them is not inconsiderable. It is too much to hope that mycologists, after reading what has been done with these obligate parasites, will be encouraged to work with some of those among the fungi. The final chapter of 76 pages by G. W. Kidder and V. C. Dewey is on the Biochemistry of Ciliates in Pure Culture. The ciliates considered are mainly Tetrahymena and most of the experimental data reported are from the work of the authors. This chapter gives a good account of the way the nutritional requirement of an organism is worked out and also indicates how much must be known before the job can be considered even nearly complete. The nutritional requirements of *Tetrahymena gelleii* are about as completely known as those of *Neurospora* and much better known than those of most of the bacteria and fungi with complex requirements. This chapter and that on the Phytoflagellates probably will interest mycologists most. The book closes with adequate author and subject indices. Some of the unfortunate arrangement of words and meaningless sentences could have been prevented by better editing by the publisher.—F. KAVANAGH (Commercial Solvents Corporation, Terre Haute, Indiana).

A DICTIONARY OF COLOR. 2ND EDITION, by A. Maerz and M. A. Paul. 208 pp. McGraw-Hill Book Company, Inc., New York, Toronto, London. 1950. Price $20.00.

Twenty years after the publication of the first edition of "Maerz and Paul" a second edition of the Dictionary of Color has appeared. Soon after its first appearance, the original edition was received with great interest by mycologists, who hoped to find a color chart useful for their purposes, mainly for a correct indication of colors of carpophores, spore deposits, color changes in the substratum caused by fungi, colors of pure cultures and their mutants, colors of chemical reactions now generally used in the Basidiomycetes, and similar descriptive purposes. Maerz and Paul was found to be quoted more and more frequently in mycological literature, and the book found a wide-open field of application in neighboring specialties such as genetics, phanerogamic botany, horticulture, etc. After a trial of nearly ten years, the reviewers found the chart useful, and in many ways superior to other frequently quoted charts for the description of carpophore and spore deposit colors in agarics although there was a marked deficiency so far as the deep and rich colors of spore deposits in Cortinariaceae, and the color of the pileus of such fungi as *Marasmius spegazzinii* (a very deep rust color) are concerned, and in grading the different shades of cream to ochraceous necessary in spore print evaluation of Russulaceae, Cantharellaceae, and other families, Maerz and Paul cannot replace the special chart published by Crawshay. The second edition has not introduced any changes in this respect. Quoting the colors is another difficulty for the mycologist who uses the book in his daily routine. The quotation as proposed by the authors themselves (e.g. 9 F 3) is insufficient unless accompanied by an explanation in words, and a quotation of the frequently fancy and not descriptive color names used by Maerz and Paul in both editions is possible only in some cases; in others, color names are not introduced, and probably rightly so. By quoting the color names, one complicates the matter for the reader of the descriptions since he has to look them up in the index before he can go to the place in the respective plate where the color is reproduced. To indicate both color name and numbers makes the quota-

tion even clumsier. It would have been a great improvement if the authors, aside from their color names and number-letter system, had introduced a system of running numbers coupled with a one-word characterization of the color such as "red 12" instead of "1 L 1." On the other hand, to the average naturalist, it is comparatively unimportant to know, as pointed out by the authors on p. 157, that "flesh" is "the usual color of human skin, female sex, face and hands excluded," and that this is "too clear and distinctly too yellowish to match any Anglo-Saxon flesh" and so on. . . . Nevertheless, mycologists began to indicate fungous colors in Maerz and Paul terms because they found a wider variety of colors matching fungous pigments, and—even more important—a better light resistance in the plates of the new chart, a feature especially advertised and, to a reasonable degree, borne out by experience. It was not necessary, as it was with Ridgway's chart, to keep the book in the dark and use it sparingly until it had, in spite of all precautions, lost its usefulness after a certain number of exposures and a certain age, with no new copies to be found in the book market.

However, comparing the new edition with the first, we find that Maerz and Paul have fallen into the same trap waiting for any second edition of books of this sort. The new plates are in many instances unlike the old ones, at least within the color ranges most frequently quoted by mycologists, the reds, browns, and yellows. Any quotations made until now—and there are hundreds in the publications of the reviewers alone—should now be revised. As an example we should like to indicate Plate 7 and Plate 8, especially the deeper shades, furthermore Pl. 16, A, C, E. Either the claims of the authors about the "permanence of colors used" (p. 1) are greatly exaggerated, and the difference between a well-kept copy of the first edition and a copy of the second edition is increased by a considerable fading of the colors, or else the new advice to specify the edition "when mentioning colors therein where extreme accuracy is desired," is merely an understatement, and should have been replaced by an admission that the new colors in many instances are far from matching those of the first edition. It is quite obvious that in science, especially in descriptive science,

reasonable permanence is required between the citation and the color in different copies of the book used; it is impossible to revise color quotations in new editions of our mycological papers every time a new edition of the color chart used by us is necessary or desirable. It is not up to us to make suggestions of a technical procedure guaranteeing such permanence, and we have to take the publisher's statement as to the difficulty of avoiding possible slight variations of color (although the variations are frequently more than slight), at its face value. We can only draw the conclusion that, if anyone has very high hopes in connection with the new edition of Maerz and Paul, he should realize that even reasonable permanence is a goal not yet achieved by any work containing color charts, and that such claims should always be understood "cum grano salis." It will perhaps be possible to expect the authors and editors to take into consideration some of the wishes and needs of descriptive natural science since, obviously, representatives of these specialties make up a large percentage of the present and future purchasers of the work.—ROLF SINGER AND ANTONIO P. DIGILIO.

MICROBIAL DECOMPOSITION OF CELLULOSE, by Ralph G. H. Siu. xi + 531 pp., 128 figs. Reinhold Publishing Co., New York. Price $10.00.

In the preface, it is suggested that this book is intended as an interpretive monograph on the fundamental phenomena involved in the microbial decomposition of cellulose. It might equally well be called an encyclopedic review. The twelve chapters include an introductory one on cellulose decomposition in nature, one on the cellulose molecule, one on the structural features of cotton fabrics, two on the morphological and taxonomic aspects of the organisms involved and two on the mechanism of degradation of fabrics, considering, respectively, gross effects and molecular transformations. The last five chapters are concerned with prevention of deterioration, covering theoretical considerations, methods of testing, use of physical barriers, of toxic inhibitors and of chemical modification. There are three general indexes, one to author citations, one to

microorganisms and one to subjects. Each chapter has its own bibliography, the number of citations for a single chapter varying from under 50 to over 500. Even allowing for duplication, the documentation is impressive, and particularly useful since it brings to light many little-known reports and other information in government files.

It is perhaps inevitable that in a work of this sort the influence of the filing card should be evident and in the volume under consideration to this is added occasional infelicities of expression which the editing of so important a book should have eliminated. The impression is given that the chemical data have been treated more critically than the biological. For example, the statement that *Memnoniella echinata* is "difficult to handle in the laboratory" (pp. 159, 336) will puzzle those who have observed its luxuriant growth on any number of common media, including fabrics. On p. 153 it is stated that cellulolytic activity has been demonstrated for eight species of Myxomycetes. Of the four references cited, one is to a thesis, two are of doubtful application, the other is to the complete set of Saccardo's *Sylloge Fungorum*, scarcely relevant in this connection, while the most convincing paper on the subject is ignored. These are minor matters, to be sure. What is important is that there is now available to the laboratory worker a comprehensive, up-to-date treatment of the phenomena of cellulose decomposition which will provide in convenient form a vast quantity of information and will serve as a help and stimulant in further research.—G. W. M.

MANUAL OF BACTERIAL PLANT PATHOGENS, SECOND, ENTIRELY REVISED EDITION (Annales Cryptogamica et Phytopathologica, Vol. 10), by Charlotte Elliott. 186 pp. Chronica Botanica Co., Waltham, Mass.; Stechert-Hafner, Inc., New York. Price $6.00.

In this edition, Miss Elliott has revised the descriptions of the earlier edition and brought the bibliography up to date. The nomenclature follows that of the 1948 edition of Bergey's Manual. Descriptions are given of 232 species and varieties of true plant pathogens in six genera: Agrobacterium, Bacterium, Corynebacterium, Erwinia, Pseudomonas, and Xanthomonas. One hundred

and three species and varieties "not recognized as valid plant pathogens" are also described. The manual should prove a valuable addition to the literature of plant pathology.—JOSEPH C. GILMAN.

THE BRITISH MYCOLOGICAL SOCIETY TRANSACTIONS. FIFTY YEAR INDEX (1896–1946; Volumes 1–30), compiled by G. R. Bisby. Introd. (unpaged) + 126 pp. Cambridge University Press. 1952. Price 21 shillings.

The importance of the contents of the *Transactions of the British Mycological Society* to mycologists the world over need not be stressed. The 50-year index will be of great help to the many who habitually consult the journal, particularly for the earlier years. The volume contains a brief introduction, publication dates of the several numbers, a record of the forays, an author index, a general index and a host index. The general index (really an index to the names of bacteria and fungi) occupies 91 pages, the bulk of the volume. New taxa are indicated by bold-face type for the pages on which they appear and figures by an asterisk.

There are a few omissions, none of which will cause difficulty to those who will read the brief introduction. Incidentally, the editor of *Mycologia* would like to have all potential contributors to that journal read, mark, learn and inwardly digest Dr. Bisby's remarks on unnecessarily long titles, to be found at the bottom of the second page of the introduction.

The British Mycological Society is to be congratulated on the completion of this significant volume. It deserves a place on all shelves where the Transactions are kept and would perhaps be even more useful in some places where they are lacking.—G. W. M.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to members of the Mycological Society of America. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

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